

**CLINICAL STUDIES OF THE RENIN-ANGIOTENSIN-
ALDOSTERONE SYSTEM AND CARDIAC
AUTONOMIC REGULATION IN MAN**

A THESIS SUBMITTED FOR THE DOCTOR OF MEDICINE DEGREE

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BY

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DECLARATION

I affirm that I am the author of this thesis. The work embodied in this thesis was performed by myself in the Department of Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee between 1st Aug 1996 and 31st July 1998. All the studies comprising this work have been published, submitted, or accepted for publication in peer-reviewed journals. The studies are linked to form a coherent body of work with a common theme. I affirm that the work is my own, and that I am solely responsible for the data analysis, interpretation, and text contained within this thesis.

I also declare that no portion of this thesis has previously been submitted for any other degree, diploma or professional qualifications at the University of Edinburgh or other institution of learning.

ABSTRACT

The work embodied in this thesis was designed to explore the interaction between the renin-angiotensin-aldosterone system (RAAS) and the autonomic nervous system. It was stimulated by the observations that the neurohormonal suppression of the RAAS by ACE inhibitors in chronic heart failure (CHF) is inadequate, and that high residual levels of circulating aldosterone have been shown to have detrimental autonomic modulating effects independent of angiotensin II in experimental models.

The effects of aldosterone blockade with spironolactone therapy were examined in CHF patients already established on ACE inhibitors. It was observed that spironolactone has beneficial parasympathomimetic properties, improving heart rate variability and reducing heart rate, particularly during the early morning hours of the day when ACTH-induced aldosterone secretion is maximal. The interaction between the RAAS and the parasympathetic tone was explored further in a series of normal volunteer studies. Although the effects of ACE inhibitors are well recognised, not much is known about the parasympathomimetic properties of direct angiotensin II or aldosterone receptor antagonism. In this thesis, it was demonstrated that losartan, an angiotensin II receptor antagonist, and enalapril, an ACE inhibitor, were equally effective in improving the vagally-mediated baroreflex response in salt depleted normotensive subjects. It was also demonstrated that direct intravenous aldosterone administration impaired the baroreflex response to vasopressor agents in healthy subjects.

The observed vagomimetic effects of aldosterone blockade may have important therapeutic implications, suggesting the possibility that spironolactone may

have anti-ischaemic or anti-arrhythmic properties. However, aldosterone blockade did not appear to have any significant impact on either autonomic tone or ischaemic events when administered to patients with ischaemic heart disease but preserved LV function. The reasons for the latter remain unclear but may reflect differences in disease-state (less neurohormonal activation, and a larger proportion of these patients was established on beta-blockers-which may influence autonomic tone – and only a minority was taking concomitant ACE inhibitors, compared to the CHF cohort). In CHF however, spironolactone was shown to improve QT dispersion, a surrogate marker of arrhythmic activity and sudden cardiac death. Mechanisms in which aldosterone may contribute towards dispersion of the QT intervals on the electrocardiogram are probably multifactorial. Aldosterone increases cardiac afterload (by increasing vascular tone and potentiating vascular smooth muscle hypertrophy) and it is demonstrated that cardiac afterload would increase QT dispersion through mechano-electrical feedback. Vagal tone modulation itself however did not contribute towards QT dispersion.

These studies demonstrate how inextricably linked the RAAS and the autonomic nervous system is. In particular, the detrimental autonomic effects of aldosterone in CHF have been highlighted. The findings of these studies highlight possible mechanisms and provide valuable insights as to why further therapeutic mileage is gained by the addition of an aldosterone antagonist in CHF patients who have already been established on ACE inhibitors.

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LIST OF ABBREVIATIONS

Ang II = Angiotensin II

BRS = baroreflex sensitivity

CHF = chronic heart failure

ECG = electrocardiogram

HF = high frequency

HRV = heart rate variability

IHD = ischaemic heart disease

LF = low frequency

LVEF = left ventricular ejection fraction

NA = noradrenaline

NYHA = New York Heart Association

QTd = QT interval dispersion

RAAS = renin-angiotensin- aldosterone system

SCD = Sudden cardiac death

CHAPTER ONE

INTRODUCTION

The past two decades have seen tremendous advances in our understanding of the renin-angiotensin-aldosterone system (RAAS) and the pathophysiology of chronic heart failure (CHF). Indeed, angiotensin-converting enzyme (ACE) inhibitors have revolutionized the management of CHF.

In recent years, ACE inhibitors have been shown to reduce the morbidity and mortality rates not only in CHF, but also in asymptomatic left ventricular dysfunction and in post-myocardial infarction patients [Cohn *et al* 1991, Pfeffer *et al* 1992, Rutherford *et al* 1994, CONSENSUS-1 1987, SOLVD 1991, AIRE 1993, Kober *et al* 1995]. However, despite these beneficial effects, the morbidity and mortality rates of CHF remains high [CONSENSUS-1 1987, SOLVD 1991]. It may however be possible to obtain more therapeutic mileage from the concept of inhibiting the RAAS than is being obtained currently in normal clinical practice. There are two main reasons for this. Firstly, the doses of ACE inhibitors currently used in clinical practice are often lower than those used in major survival trials [Cleland and Poole-Wilson 1995]. These low doses used in clinical practice may not be optimally effective and higher doses may be required to inhibit the RAAS optimally. High doses of ACE inhibitors appear to suppress the RAAS more effectively than low doses [Davidson *et al* 1996]. Secondly, even when used at the highest tolerated doses, ACE inhibitors themselves may not completely suppress the RAAS. In a significant proportion of these patients on chronic ACE inhibition, the plasma aldosterone (and to a lesser extent, Angiotensin II [Ag II]) levels rise again despite initial suppression, after as little as three months of treatment [Staesson *et al* 1981, Barghi *et al* 1993]. There is a growing body of evidence to suggest that the neurohormonal re-activation, especially

that of aldosterone, has adverse effects on the cardiovascular system, such as the modulation of the cardiac autonomic tone. In fact, aldosterone blockade has recently been shown to reduce both morbidity and mortality among severe CHF patients already established on ACE inhibitors [Pitt *et al* 1999]. These observations form the core of this thesis. The potential adverse effects of the neurohormonal “escape” of the RAAS axis despite ACE inhibitors, are explored in the studies carried out in this thesis.

By way of an introduction, I shall first of all, present an overview of the RAAS axis, with particular attention paid to the potential mechanisms and clinical significance of the neurohormonal reactivation despite ACE inhibitors in CHF. Whereas the adverse effects of Ang II are well documented, the potential adverse effects of aldosterone on the cardiovascular system are less well known. The current literature on the role of aldosterone escape in CHF is also reviewed, highlighting where relevant, the unresolved issues which prompted the studies constituting this thesis.

1.1 *Overview of the Renin-Angiotensin-Aldosterone System and its inhibition: Evidence from the large mortality trials*

The organisation the RAAS system is shown in *Fig 1.1*. In low cardiac output states like heart failure, there is activation of the RAAS system, which operates in concert with the sympathetic nervous system to maintain arterial pressure. Firstly, stimulation of the β 1-adrenoceptors in the juxtaglomerular apparatus of the kidneys as a result of heightened adrenergic drive is a principal mechanism responsible for the release of renin in CHF. Secondly the reduced cardiac output and vasoconstriction of

heart failure lead to a redistribution of blood flow away from the kidney. The decreased renal perfusion and the subsequent activation of the baroreceptors in the renal vascular beds also increase renin secretion. Plasma renin activity is further increased in CHF by diuretic therapy and salt restriction (which result in a reduction of sodium presented to the macula densa), and activation of left atrial volume receptors.

The increased plasma renin activity leads to the formation of Ang II. Among the components of the RAAS, Ang II has the most potent and best-studied effects (see *Table 1.1*). It is a potent vasoconstrictor, up to 40 times stronger than noradrenaline, and contributes to the excessive elevation of systemic vascular resistance in CHF. In addition Ang II increases sympathetic outflow. It enhances peripheral sympathetic transmission; it augments output of norepinephrine from nerve terminals and block reuptake of norepinephrine. It also stimulates vasopressin and aldosterone release.

Aldosterone secretion is regulated by the RAAS system, potassium ion and ACTH. It has potent sodium retaining properties. Renin and aldosterone secretions respond dynamically to even small changes in sodium balance. Aldosterone is also a primary regulator of potassium balance, providing homeostasis over a wide range of variation in potassium intake. In addition to these well known renal effects, there is now increasing evidence emerging from animal studies and clinical observations that aldosterone may have other adverse direct cardiac and vascular effects. Recent experiments have uncovered aldosterone binding sites in the heart, vascular wall and the brain in addition to the well-known renal receptors in the distal nephron. These potentially harmful effects are reviewed separately in *Sections 1.3-1.6*.

It is clear by looking at the adverse effects of Ang II and aldosterone, that there is much to be gained by neurohormonal suppression of the RAAS in CHF, which would reduce systemic vascular resistance, diminish afterload and improve cardiac output. Indeed a number of large well-designed randomised trials have demonstrated that ACE inhibitors improve survival in these patients regardless of aetiology or severity of symptoms. The Cooperative Northern Scandinavian Enalapril Survival Study [CONSENSUS-1 1987] demonstrated a 40% reduction in mortality at 6 months in patients with severe heart failure who were randomised to enalapril rather than placebo. Subsequent trials have examined the effects of ACE inhibitors in mild or moderate heart failure. The treatment arm of the Studies on Left Ventricular Dysfunction trial [SOLVD 1991] that randomised patients with symptomatic mild-to-moderate heart failure with left ventricular ejection fraction (LVEF) less than 35% to either enalapril or placebo reported a statistically significant 16% reduction in overall mortality in the enalapril treated group.

The second Veterans Administration Cooperative Vasodilator Heart Failure Trial (V-HeFT-II) showed a small but clear survival benefit in patients with mild-to-moderate heart failure who had been randomised to receive enalapril rather than the combination of hydralazine and isosorbide dinitrate [Cohn *et al* 1991]. The Survival and Ventricular Enlargement (SAVE) trial studied patients with recent myocardial infarction and LVEF of 40% or less, but without overt heart failure, and showed a 20% reduction in mortality and a 36% reduction in the rate of progression to severe heart failure in the captopril-treated group after 48 months of follow up [Pfeffer *et al* 1992, Rutherford *et al* 1994]. Both the SOLVD [1991] and the SAVE [Pfeffer *et al*

1992, Rutherford *et al* 1994] trials demonstrated that enalapril and captopril, respectively, markedly reduced or prevented the increases in left ventricular end-diastolic and end-systolic volumes and decline in EF observed in patients randomised to receive placebo. The Acute Infarction Ramipril Efficacy trial was similar to the SAVE trial, except that it randomised only patients who manifested clinical evidence of heart failure to either ramipril or placebo [AIRE 1993]. There was a significant 27% reduction in mortality in the ACE inhibitor treated group that was apparent within 30 days of treatment (unlike SAVE, in which the survival curves did not diverge until 1 year). The Trandolapril Cardiac Evaluation (TRACE) trial studied patients with severe left ventricular dysfunction 3 to 7 days following myocardial infarction and reported a 20% reduction in mortality [Kober *et al* 1995].

Taken together these trials indicate that ACE inhibitors prolong survival in a broad spectrum of patients with myocardial infarction and heart failure, ranging from those who are asymptomatic with LV dysfunction to those with symptomatic heart failure. However, despite these major advances, a considerable degree of mortality still remains eg. 36% in CONSENSUS-1 (severe CHF) after 1 year, and 35% in SOLVD (mild to moderate CHF) after 4 years. Similarly whereas hospitalisation in SOLVD decreased by 15%, a considerable number of hospitalisations for heart failure remained. The reasons for these high morbidity and mortality rates are unclear. The beneficial effects of ACE inhibitors have been attributed to its ability to cause neurohormonal suppression. For example, in the V-HeFT II Study [Cohn *et al* 1991], enalapril had a better effect on survival than did the combination of hydralazine and isosorbide dinitrate. Whereas both treatments cause vasodilation, only enalapril causes neurohormonal suppression. Furthermore, in the CONSENSUS I Study, increased

levels of Ang II and Aldosterone correlated with mortality [Swedberg *et al* 1990]. However the long term suppressive effects of ACE inhibitors on aldosterone are weak, variable and unsustained [Struthers 1996]. Therefore, neurohormonal reactivation or “escape” of aldosterone (and to a lesser extent, Ang II) as mentioned earlier, may be one of the reasons why mortality rates remain high in conditions such as CHF despite ACE inhibitor therapy.

The effects of concomitant administration of the aldosterone antagonist, spironolactone, with ACE inhibitors were recently evaluated in the multicentre Randomised Aldactone Evaluation Study (RALES) [Pitt *et al* 1999]. In this randomised double-blind study, 1663 CHF patients with an LV ejection fraction <35% and who were already established on ACE inhibitors were assigned to either spironolactone 25mg daily or placebo. Over a mean follow up period of 24 months, both total mortality (including sudden deaths) and hospitalisations were substantially reduced by 30% and 35% respectively, in the spironolactone group compared to placebo. These results suggest that neurohormonal suppression by ACE inhibitors alone may not be adequate and further therapeutic mileage may be obtained through the addition of an aldosterone antagonist.

1.2 Mechanisms for neurohormonal reactivation (escape) during chronic ACE inhibition

The introduction of ACE inhibitor therapy reduces plasma Ang II and aldosterone levels in CHF. It is however uncertain whether the initial reduction in plasma aldosterone levels promoted by ACE inhibition can be sustained. A resurgence in aldosterone (and to a lesser extent, Ang II) levels has been observed after as little

as 3 months of ACE inhibitor therapy in hypertension [Staesson *et al* 1981, Biollaz *et al* 1982]. A similar pattern can also be seen with the use of ACE inhibitors following myocardial infarction [Barghi *et al* 1993]. In CHF, Cleland *et al* [1984] found that a wide and variable range of plasma aldosterone levels remained after 6 weeks of treatment with captopril.

There are several possible underlying mechanisms for this aldosterone escape phenomenon. Firstly, aldosterone escape may be *secondary* to angiotensin II reactivation during chronic ACE inhibition. Secondly, even when Ang II production is completely suppressed, there is evidence to suggest that aldosterone escape may occur *independently*. Staessen *et al* [1981] found an increase in plasma aldosterone levels after 3 months of ACE inhibition that was *not* associated with an increase in plasma levels of Ang II. This suggests that aldosterone and Ang II levels may become dissociated over time and raises the possibility that there are alternative pathways for aldosterone production.

I will now discuss some of these mechanisms.

Angiotensin II reactivation:

Ang II reactivation may be the result of any, or all, of the following:

- i) Poor compliance with ACE inhibitor therapy. Non-compliance is more likely in patients with CHF as they are elderly and often on multiple drug therapy [Monane *et al* 1994].
- ii) Counteracting effects of concomitant treatment with diuretics and vasodilators [Doig *et al* 1993]. Vasodilators such as nitrate-hydralazine [Cohn *et al*

1991], calcium channel blockers [Packer 1989] and diuretic-induced salt and water loss [Bayliss *et al* 1987, Kubo *et al* 1987, Cleland *et al* 1988] have all been shown to cause neurohormonal activation.

iii) Generation of Ang II by a non-ACE dependent pathway. The first physiological descriptions of alternative Ang II formation were reported by Cornish *et al* [1979] in the hamster cheek pouch, and by Trachte and Lefer [1979] in the cat cardiac papillary muscle. Cornish *et al* [1979] also found ACE-independent Ang II formation in the hamster coronary artery. Since then numerous investigators have reported ACE inhibitor-insensitive Ang II formation in the heart, blood vessels or systemic circulation in different species including man [Urata *et al* 1990, Okunishi *et al* 1984, Chen *et al* 1991]. Urata *et al* [1990] reported a dual pathway for Ang II formation in the human heart *in vitro* where the chymase enzyme is the main enzyme involved in Ang II formation, in addition to the ACE enzyme system. Chymase or other endopeptidases can convert Ang I to II without being inhibited by ACE inhibitors. Whether they are present in sufficient quantity to generate significant amounts of Ang II either systemically or at a local (tissue) level is uncertain. Studies suggest that mRNA for chymase is expressed at much lower levels than mRNA for ACE in human cardiac tissues [Morgan *et al* 1994]. Several other enzymes such as chymotrypsin angiotensin generating enzyme (CAGE) and cathepsin D can also produce Ang II from Ang I *in vitro*, but again their physiological roles in the cardiovascular system *in vivo* have not been clarified [Morgan 1993].

In summary, ACE inhibition appears to be a relatively non-specific approach to blockade of the RAAS system. It is likely that a more complete blockade can be

achieved by blocking the Ang II receptor directly with an angiotensin II type 1 (AT1) receptor antagonist. Specific blockade of the Ang II receptor has other theoretical advantages over nonspecific ACE inhibition. Unlike ACE inhibitors, which inhibit bradykinin degradation, AT1 receptor antagonists such as losartan do not increase bradykinin levels. Hence AT1 receptor antagonists lack the bradykinin-related adverse effects profile of ACE inhibitors such as cough and angio-oedema. However the decrease in breakdown of bradykinin may also be partly responsible for some of the beneficial effects seen with ACE inhibitors. Not much is known about bradykinin; it is associated with the release of nitric oxide and prostacyclin, which may contribute to the haemodynamic effects of ACE inhibitors [Gavras 1992]. Therefore although AT1 receptor antagonists may provide a more complete blockade of Ang II along with a better side-effect profile, it may potentially lose out in terms of the benefits resulting from the increased bradykinin levels found with ACE inhibitors. To fuel the controversy even further, it is now also thought that AT2 receptors stimulation may increase local bradykinin levels (although not as much as ACE inhibitors).

The Evaluation of Losartan in the Elderly (ELITE) trial [Pitt *et al* 1997] was the first long term trial (48 weeks) to compare an ACE inhibitor, captopril, with an AT1 receptor antagonist, losartan in CHF patients with EF<40%. In this study, those randomised to losartan had a 46% reduction in all-cause mortality in comparison to captopril-treated patients, which was primarily due to a decrease in sudden cardiac deaths. However the study was only designed to assess safety and efficacy of the treatments; mortality was not the primary endpoint and the absolute number of deaths was small. The intriguing results of ELITE were however not replicated in a subsequent larger scale randomised trial in which total mortality was the primary

endpoint (ELITE II). In the latter study, , although losartan was significantly better tolerated, it did not appear to be superior to captopril in improving survival in CHF patients. At present, there are clearly, many unresolved issues regarding the use of AT1 receptor antagonists in CHF. Increased sympathetic tone and impaired baroreceptor function are key processes, which predispose the myocardium to sudden cardiac death in CHF. At present, no one knows if AT1 receptor antagonists attenuate the cardiac autonomic tone more efficaciously than ACE inhibitors. This is one of the issues that will be addressed in further detail in this thesis.

Aldosterone reactivation:

Aldosterone escape during chronic ACE inhibition may occur through several mechanisms. It may be:

i) secondary to Ang II reactivation.

ii) it may be produced by non-Ang II dependent mechanisms regulated by corticotropins, atrial natriuretic peptide and serum potassium [Weber *et al* 1993]. Certainly when Ang II production is completely suppressed, aldosterone escape may still occur [Staesson *et al* 1981].

iii) a result of impaired aldosterone clearance. Aldosterone is extracted almost exclusively from the splanchnic circulation and metabolised in the liver. In patients with CHF, in whom hepatic and splanchnic blood flows are impaired, splanchnic extraction and clearance of aldosterone are reduced [Tait *et al* 1965].

This aldosterone escape may affect the myocardium adversely in a number of ways. In CONSENSUS [1987], baseline levels of aldosterone were predictive of long-term survival in placebo recipients, and patients with more complete aldosterone

suppression among ACE inhibitor recipients had better survival outcome than those with less effective suppression. In addition to its well known renal effects (sodium and water retention, hypokalemia and hypertension) aldosterone is now thought to have adverse autonomic effects not unlike those of Ang II. Furthermore, aldosterone may promote myocardial fibrosis, electrolyte disturbances and predispose the myocardium to ventricular arrhythmias.

The evidence for these effects of aldosterone are reviewed in the following sections.

1.3 *Aldosterone and autonomic regulation in CHF*

In CHF, the RAAS and the autonomic nervous system are inextricably linked. It is well established that Ang II attenuates the baroreflex control of heart rate and sympathetic activity [Guo and Abboud 1984, Mace *et al* 1985]. Activation of the sympathetic nervous system, RAAS and parasympathetic withdrawal occur in response to the inadequate “filling” of the arterial tree which is characteristic of systolic heart failure. In the early stages of acute systolic failure, these changes are compensatory and act to maintain perfusion to vital organs and to expand the inadequate arterial blood volume. However, as heart failure becomes chronic, these compensatory mechanisms can cause undesirable effects such as excessive vasoconstriction, increased afterload, excessive salt and water retention, electrolyte abnormalities and arrhythmias. Patients with CHF have depleted myocardial catecholamine stores [Chidsey *et al* 1965] and down regulated beta-receptors [Bristow *et al* 1982], and these abnormalities may further contribute to the demonstrated inability of the cardiovascular system to respond to stress in heart

failure.

Mechanisms for sympathetic activation and parasympathetic withdrawal in heart failure

Sympathetic activation and parasympathetic withdrawal in heart failure have been attributed to alterations in inhibitory and excitatory influences on brainstem vasomotor neurons.

Excitatory inputs on brainstem vasomotor neurons are derived from arterial chemoreceptors and muscle “metaboreceptors”. Baroreceptor afferents from the carotid and aortic arch (arterial high pressure mechanoreceptors) and the cardiopulmonary regions (low pressure mechanoreceptors) on the other hand, are the principal inhibitory influences on vagal and sympathetic outflow to the heart and circulation. Sensory information from the mechanosensitive endings in the carotid sinus and aortic arch travels to the central nervous system by means of the glossopharyngeal and vagal nerves, respectively. The primary afferent neurons synapse in the nucleus tractus solitarius, from which there are projections to the medulla and other regions of the brain stem. Increases in arterial pressure activate these primary afferent neurons and result in increased vagal efferent outflow and reduced sympathetic outflow to the heart and circulation. Vagal efferent outflow originates from neurons in the nucleus ambiguus, whereas sympathetic neural influences are controlled from the rostral and caudal ventrolateral medulla and project to the intermediolateral cell column, which is the site of origin of the preganglionic cell bodies of the sympathetic nerves. These preganglionic cell bodies project to the sympathetic ganglia, including the stellates, which are the sites of origin of the

postganglionic sympathetic fibers. Similarly, sensory information from the receptors in the cardiopulmonary region (located throughout the heart and lungs) travels over the vagal nerves to the nucleus tractus solitarius and exerts influences on the vagal and sympathetic outflow.

Under normal conditions, the inhibitory inputs dominate, and the integrated response to these competing influences results in low efferent sympathetic traffic and arterial catecholamines, and high heart rate variability.

As heart failure progresses, the principal stimuli to arterial baroreceptor afferent discharge (eg. systolic blood pressure, rate of rise of blood pressure etc) become blunted, and the sensitivity of the mechanoreceptors to stretch diminishes, resulting in decreased inhibitory input from the arterial and cardiopulmonary receptors [Hirsch *et al* 1987, Ferguson 1990, Wang *et al* 1990, Hainsworth 1991] and increased excitatory inputs [Ferguson 1990]. The net response to this shift in balance between the inhibitory and excitatory afferent inputs includes a generalised increase in basal sympathetic outflow, parasympathetic withdrawal, blunted reflex parasympathetic (ie. impaired baroreceptor sensitivity) and sympathetic control of heart rate (reduced heart rate variability), and impairment of the reflex sympathetic regulation of vascular resistance [Hirsch *et al* 1987, Ferguson *et al* 1991, Saul *et al* 1988, Casolo *et al* 1991].

Circadian variation of autonomic regulation in CHF

The integrated response to these neural influences is a dynamic process undergoing continuous but predictable changes throughout day and night in normal man. These circadian fluctuations of the cardiac autonomic tone are best reflected by

analysis of heart rate variability (HRV) [Saul *et al* 1988, Casolo *et al* 1991].

HRV can be analysed as a function of time using parametric indices of measurement (time domain analysis) or by exploration of the periodic oscillations of heart rate at different frequencies using spectral frequency domain analysis (see *Chapter 2: Methods*). In power spectral analysis, there are three main spectral components: very low frequency (VLF), low frequency (LF) and high frequency (HF) components. Vagal activity is the major contributor to the HF component. However controversy exists with regards to the LF component - whereas some studies suggest that LF, when expressed in normalised units, is a quantitative marker of sympathetic modulation, other view LF as reflecting both sympathetic and vagal activity. Consequently, the LF/HF ratio is considered by some to mirror sympathovagal balance or to reflect the sympathetic modulation [Task Force of the ESC 1996]. The physiological explanation for the VLF component is much less defined.

Spectral analysis of 24hr recordings have shown consistently that in normal subjects, LF and HF exhibit a circadian pattern and reciprocal fluctuations with higher values of LF in the daytime and of HF at night, corresponding with a sympathetic predominance during the day and vagal predominance during the night [Furlan *et al* 1990]. A major change in the autonomic profile occurs at approximately 6am, coinciding with the time of awakening, when a surge of sympathetic activity occurs rapidly and concomitantly with vagal withdrawal [Furlan *et al* 1990].

In CHF, this normal circadian variation of the autonomic tone is disrupted. Both time-domain and frequency-domain parameters of HRV are reduced [Casolo *et al* 1991, Casolo *et al* 1989, Panina *et al* 1995, Adamopoulos *et al* 1995, Tani *et al* 1991]. Data on the circadian variations of the spectral components are however

limited and conflicting in CHF. Casolo *et al* [1991] and Panina *et al* [1995] both reported reduced HRV in CHF patients but no significant changes in any of the spectral components were observed over a 24hr period. Adamopoulos *et al* [1995] on the other hand observed a reduced but still preserved circadian pattern in the spectral components. Whilst Tani *et al* [1991] found that LF and HF components were significantly reduced at any time compared to HRV in normal subjects, they observed a loss of the circadian variation of the LF component but the 24hr variation of the HF component was preserved.

It must be borne in mind that these spectral HRV indices are not direct measures of autonomic tone, since by their nature they reflect the power of an oscillation, but not the absolute level of the signal [Malik and Camm 1993]. For instance, the LF component which reflects sympathetic activity, is diminished with worsening heart failure, and this is thought to be due to extreme adrenergic hyperactivity, leading to saturation of the beta-receptors, limited oscillations and an incapacity to respond to modulating mechanisms [Malik and Camm 1993]. This abnormal circadian regulation of the autonomic tone in CHF may be an important factor contributing to the high incidence of adverse cardiac events such as lethal ventricular arrhythmias. Certainly, acute ischaemic events [Muller *et al* 1985, Goldberg *et al* 1990, Hansen *et al* 1992], arrhythmic events [Raeder *et al* 1988, Mallavarapu *et al* 1995, Davila *et al* 1995] and sudden cardiac deaths [Willich *et al* 1987, Levine *et al* 1992, Marron *et al* 1994, Moser *et al* 1994] are all known to exhibit a circadian pattern with an increased incidence during the morning hours compared to the evening period (See *Section 1.7*).

Potential autonomic modulating properties of aldosterone:

Recent experimental work suggest that aldosterone possess autonomic modulating effects independent of Ang II. Firstly, aldosterone may potentiate the effects of catecholamines. Aldosterone has been shown to block myocardial uptake of norepinephrine *in vivo* in an animal model [Barr *et al* 1995]. In support of this experimental finding, in a clinical study with CHF patients, the addition of spironolactone, an aldosterone antagonist, was found to increase myocardial norepinephrine uptake by metaiodobenzylguanidine (MIBG) scanning [Barr *et al* 1995].

Secondly, aldosterone may also possess direct constrictor effects on the vascular smooth muscle. This may be the biologic consequence of aldosterone's potentiation of catecholamines [Weber and Purdy 1982]. Furthermore, aldosterone receptors and mRNA encoding the key enzyme for aldosterone biosynthesis have also been detected in both endothelial and smooth muscle cells from the human pulmonary artery [Hatakeyama *et al* 1994]. Endothelium-dependent vasodilation by acetylcholine is reduced in patients with essential and renovascular hypertension as well as in patients with primary aldosteronism compared to control subjects; surgical correction of the aldosteronism, on the other hand, restored endothelial function [Taddei *et al* 1993]. These data suggest that endothelium-dependent vasodilatation by acetylcholine is attenuated by high aldosterone levels. Schohn *et al* [1993] observed that spironolactone attenuated the haemodynamic effects of intravenously infused Ang II and noradrenaline in anuric terminal renal failure patients, suggesting that the effects of aldosterone antagonism was extra-renal, and probably through a direct vascular reffect.

In addition to sympathetic potentiation, current evidence also suggest that aldosterone may reduce parasympathetic activity. Wang *et al* [1992] showed conclusively that aldosterone not only directly reduces baroreceptor discharge from the carotid sinus in an animal model, but also that aldosterone reduces the heart rate response to changes in blood pressure. These effects were seen with both acute and chronic administration of aldosterone [Wang 1994]. In man, aldosterone infusion had been shown to halve the bradycardic response to infused noradrenaline, a vasopressor agent [Barr and Struthers 1994]. Furthermore, MacFadyen *et al* [1997] had recently found that spironolactone improved heart rate variability (an index of parasympathetic activity) in CHF patients. An interesting observation from this study is the fact that spironolactone reduced the 6-9am increase in heart rate in these patients. As aldosterone under the influence of ACTH secretion, is known to peak at this time of the day, as does cortisol [Armbruster *et al* 1975], it is perhaps not surprising that the autonomic effects of spironolactone are most prominent at 6-9 am.

These studies suggest that the ACTH induced dawn increase in aldosterone may alter the autonomic balance in such a way as to increase ischaemia, arrhythmias and sudden death.

1.4 The arrhythmogenic role of Aldosterone:

There are a number of reasons why aldosterone is potentially arrhythmogenic. This may be the result of:

i) augmentation of the autonomic nervous system: As mentioned in the previous section, aldosterone may potentiate the action of catecholamines and reduce

parasympathetic activity. Whereas catecholamines are arrhythmogenic, the parasympathetic system has cardioprotective effects. For example, in animal models of myocardial ischaemia, vagal stimulation reduced the frequency of reperfusion induced VF from 60% to 7% while abolishing ventricular tachycardia altogether in ischaemic cats [Zuanetti *et al* 1987], whereas in ischaemic dogs, vagal stimulation had been shown to increase survival from 12% to 57% [Myers *et al* 1974]. Hence by reducing heart rate variability and baroreflex sensitivity - both surrogate markers for sudden cardiac death [Barron and Lesh 1996]- aldosterone may play a pivotal role in the generation of malignant arrhythmias and sudden death in cardiovascular diseases.

ii) magnesium and potassium depletion: Aldosterone independently increases urinary magnesium and potassium loss [Rahman *et al* 1992]. Both hypokalemic and hypomagnesaemic states increase cell excitability [Whang and Welt 1963] and are potentially arrhythmogenic [Fisch 1973, Gettes 1976]. A low serum magnesium concentration is associated with an increase in ventricular ectopy in CHF [Eichhom *et al* 1993]. Conversely, magnesium replacement causes a decline in the number of ventricular arrhythmias in CHF [Gottlieb *et al* 1990, Bashir *et al* 1993]. In addition to direct electrophysiological effects [Kaseda *et al* 1989], magnesium depletion may also increase the arrhythmogenic response to catecholamines [Bean and Varghese 1994]. Although a clear link is established between magnesium depletion and arrhythmias, the effect of magnesium on mortality is uncertain. Although intravenous magnesium had no effect on mortality in acute myocardial infarction (where a large proportion of the patients are not on diuretic therapy) [ISIS-4 Group 1995], the situation may be different in CHF, where the patients are on chronic therapy with magnesium and potassium losing diuretics .

iii) *myocardial fibrosis*: Chronic administration of aldosterone induced biventricular fibrosis in the rat [Brilla and Weber 1992]. This myocardial fibrosis could be prevented by spironolactone at a dosage too low to alter blood pressure [Brilla and Weber 1992], suggesting that the mechanisms in which aldosterone produce fibrosis are independent of its effect on blood pressure and left ventricular hypertrophy. Aldosterone has been shown to induce mRNA for collagen formation in both left and right ventricles [Robert *et al* 1994]. MacFadyen *et al* [1997] recently provided further evidence by showing that aldosterone blockade with spironolactone reduced serum procollagen type III amino terminal peptide (PIIINP), a marker of collagen turnover, in CHF patients. Patchy myocardial fibrosis may cause electrical inhomogeneity and reduce threshold for ventricular fibrillation [Pye and Cobbe 1992].

iv) *direct arrhythmogenic effect*: In an animal model by Arora and Somani [1962], the direct administration of aldosterone infusion into coronary artery ligated dogs was associated with the generation of ventricular arrhythmias. This pro-arrhythmic effect of aldosterone was not only dose-dependent, but it was more striking and prolonged compared to the arrhythmogenic agent, epinephrine. As the arrhythmogenic effect of the aldosterone infusion was of rapid onset, electrolyte depletion was an unlikely mechanism for this phenomenon.

In conclusion, aldosterone may produce arrhythmias through a combination of any or all, of the above mechanisms. High levels of aldosterone in conditions such as CHF may therefore contribute to the development of malignant ventricular arrhythmias and the high incidence of sudden death.

1.5 Aldosterone and QT dispersion:

Recently, QT dispersion (QTd), which is the difference between the longest and shortest QT interval measured on the 12-lead electrocardiogram (ECG), has been identified as a simple and easily measured predictor of sudden cardiac death [Barr *et al* 1994, Molnar *et al* 1997]. Although not fully understood, QTd is thought to be a measure of electrical inhomogeneity in the heart which decreases an individual threshold for ventricular arrhythmias [Pye and Cobbe 1992]. Han and Moe [1964] showed that differences in refractory periods in adjacent areas could be responsible for the occurrence of arrhythmias especially fibrillation. This spatial inhomogeneity of the repolarisation phase of the action potentials may be caused by either different durations of the action potentials, or by action potentials arriving late due to slow conduction at different sites of the myocardium (eg, ischaemic or fibrotic areas).

At present there are still many gaps in our understanding as to what causes these differences in action potential durations in ventricles. Although the effect of aldosterone on QTd has not been documented, there are however, several potential mechanisms by which hyperaldosteronism may contribute to increased QTd in conditions such as CHF. In fact, most of the adverse effects of aldosterone described in the previous sections could potentially increase QTd.

Firstly, current evidence suggests that aldosterone has particularly malignant autonomic effects (potentiating sympathetic activity and reducing parasympathetic tone) and that these may contribute to increased QTd [Bonnar *et al* 1997]. The significance of these neural influences is further supported by the observations that the only drugs so far to have reduced QTd are those that modulate the autonomic tone,

such as beta-blockers [Priori *et al* 1994, Day *et al* 1991] and ACE inhibitors [Barr *et al* 1997], which are well recognised to have vagomimetic effects [Flapan *et al* 1992, Osterziel *et al* 1996].

Secondly, QTd could also be due to patchy myocardial fibrosis, promoted by hyperaldosteronism [MacFadyen *et al* 1997, Brilla and Weber 1992], which leads to electrical inhomogeneity and anisotropic re-entry arrhythmias [Pye and Cobbe 1992, Han and Moe 1964]. Thirdly, QTd is linked to ventricular dilatation [Pye *et al* 1994]. Aldosterone may cause dilatation of the ventricles through a variety of mechanisms: by increasing afterload (through increased vascular tone, salt and water retention and autonomic modulation), and promoting myocardial fibrosis. Fourthly, there is some evidence to suggest that aldosterone may also have ischaemic enhancing properties (see *Section 1.6*). Janse *et al* [1985] found that myocardial ischaemia and infarction increase the local dispersion in animal models mainly by a decrease in conduction velocity and to a lesser extent, by action potential differences. Furthermore, repeated ischaemic episodes and infarctions predispose the myocardium to scarring and patchy fibrosis. In agreement with this, recent studies have confirmed that ischaemia increases QT dispersion [Sporton *et al* 1997].

Finally, electrolyte depletion, in particular that of potassium and magnesium, may also contribute to dispersion of the QT intervals. Both hypokalemic and hypomagnesaemic states are arrhythmogenic, associated with prolongation of the QT intervals and increased likelihood of developing ventricular arrhythmias such as torsade de pointes [Whang and Welt 1963, Fisch 1973, Gettes 1976]. Conversely, magnesium replacement therapy shortens the QT interval and has been shown to be a very effective anti-arrhythmic agent in terminating episodes of torsade de pointes

[Tzivoni *et al* 1984]. The effects of magnesium on QT dispersion is at present unknown. Potassium, on the other hand, is one of the main determinants of the QT interval, as it is responsible for the outward repolarisation currents. Reduction of serum potassium, results in slower repolarisation and prolonged QT intervals [Nabauer *et al* 1993]. Recently, Choy *et al* [1997] observed that intravenous potassium infusion not only normalised QT prolongation but also reduces dispersion in CHF and in normal subjects pre-treated with quinidine. The relationship between potassium and QT dispersion may have important clinical implications particularly in CHF, where QT dispersion is increased and potassium levels are chronically deficient as a result of hyperaldosteronism and diuretic therapy. In addition to CHF, these observations may also be of relevance in other clinical settings. For instance, recent observations suggest that hyperinsulinaemic states are associated with increased QT dispersion [Watanabe *et al* 1997]. The underlying mechanisms are unclear but once again, potassium may play a significant role contributing to QT dispersion as hyperinsulinaemia causes hypokalemia by pushing potassium into cells.

In summary, aldosterone can cause QT dispersion through a number of ways. Some of these mechanisms are explored further in this thesis. They have important clinical and therapeutic implications as QT dispersion is a predictor of sudden death in a variety of clinical settings [Barr *et al* 1994, Molnar *et al* 1997, Buja *et al* 1993, Potratz *et al* 1993, Perkiomaki *et al* 1995, Darbar *et al* 1996].

1.6 Aldosterone and myocardial ischaemia

Although there is no direct evidence that aldosterone causes myocardial

ischaemia, there are a number of interesting observations. Aldosterone blockade has been shown to reduce the dawn (6-9am) increase in heart rate in CHF patients [MacFadyen *et al* 1997]. Spironolactone may therefore have anti-ischaemic effects by way of reducing heart rate at this time. The importance of this observation is that this is the same time of day when ischaemic, arrhythmic and sudden death events are commonest (see *Section 1.7*).

Aldosterone may cause an increase in myocardial oxygen demand through a number of ways, including potentiation of sympathetic activity and withdrawal of vagal cardioprotection (*Section 1.3*). The increase in sympathetic activity may also increase coronary artery tone [Weber and Purdy 1982, Hatakeyama *et al* 1994, Taddei *et al* 1993]. Aldosterone-induced magnesium depletion could also cause coronary vasoconstriction [Turlapaty and Altuna 1989]. Aldosterone blockade has also recently been shown to improve endothelial dysfunction and increase vascular nitric oxide bioactivity in CHF patients [Farquharson and Struthers 2000]. Finally, aldosterone itself may be implicated in the pathogenesis of atherosclerosis. Patients with primary hyperaldosteronism have endothelial dysfunction, which is reversed when the tumour is removed [Taddei *et al* 1993]. Furthermore monocytes which are implicated in the development of atherosclerosis, appear to have functionally active aldosterone receptors [Wehling *et al* 1987].

1.7 The circadian variation of aldosterone and cardiovascular events:

Like cortisol, aldosterone secretion follows a diurnal variation, with peak secretion during the morning hours [Armbruster *et al* 1975]. In CHF, this ACTH-

induced dawn surge in aldosterone still occurs despite ACE inhibitors therapy [Davidson *et al* 1996]. This diurnal profile mirrors that of cardiovascular events.

Numerous epidemiologic studies have shown that the frequency of acute cardiovascular events is not evenly distributed throughout the day, with a peak incidence occurring during the morning hours (6am - 12 noon), after a nadir in these events at nights. Muller *et al* [1985] who were the first to establish the circadian pattern of acute myocardial infarction, found that AMI was at least 3 times more likely to occur in the morning than in the late evening. Since then, a number of studies [Goldberg *et al* 1990, Hansen *et al* 1992] have confirmed these observations by analysing detailed records of patient symptoms and the time-activity patterns of cardiac enzymes. Studies evaluating 24hr to 48hr Holter ambulatory ECG in patients with stable angina have shown that transient myocardial ischaemic events too exhibit a circadian pattern [Rocco *et al* 1987].

The distribution of ventricular arrhythmias over 24hrs has also been observed to conform to a similar diurnal pattern. Raeder *et al* [1988] observed a striking diurnal distribution of ventricular ectopic activity with a trough at 5 am and a peak between 10 am and noon, in patients with a history of malignant sustained ventricular tachyarrhythmias. This finding is supported by recent studies of patients with implantable cardioverter-defibrillator where ventricular arrhythmias recorded by the device have been shown to exhibit a circadian pattern with occurrence rate lowest between 2am and 3 am and highest between 10am and 11 am [Mallavarapu *et al* 1995]. Implantable defibrillator shocks were delivered mostly during the morning hours [Davila *et al* 1995].

Similarly, the circadian pattern of sudden deaths have been documented in

patients with previous myocardial infarction [Willich *et al* 1987, Levine *et al* 1992], hypertrophic cardiomyopathy [Maron *et al* 1994] and CHF [Moser *et al* 1994]. Moser *et al* [1994] found that in patients with advanced CHF (NYHA III/IV) 46% of deaths occurred between 6 am and noon, accounting for a 2.5 fold increase in the risk of sudden death in the morning hours compared with the other times of the day.

The mechanisms underlying the circadian pattern of adverse cardiac events are not known. Further characterisation of this circadian pattern has identified the hours of awakening, rather than the hour of the day as being most closely related to the occurrence of adverse events [Willich *et al* 1992, Muller *et al* 1989]. Willich *et al* [1992] found that after adjusting for individual waking times, the relative risk of sudden cardiac death was 2.6 during the first 3 hours after awaking when compared to the rest of the 24hr period. Furthermore this morning peak of cardiovascular events is known to coincide with the surge of sympathetic activity and vagal withdrawal, as reflected by changes in various autonomic and physiological parameters such as increased heart rate and blood pressure, raised plasma catecholamines, increased vascular tone and enhanced platelet aggregability. As discussed in *Section 1.3*, the sympathetic tone is known to exhibit a circadian rhythm characterised by diminished activity during the night and a peak in the morning hours associated with assumption of the upright position. Diabetic patients with autonomic dysfunction [Zarich *et al* 1994] and patients on beta-blockers [Peters 1990] who do not demonstrate this morning peak of events provide indirect evidence that alterations in the sympathovagal balance may be partly responsible for the circadian pattern of cardiovascular events.

Aldosterone secretion may play a contributory role to the development of the diurnal pattern of these adverse events, especially in view of the growing body of evidence that aldosterone has detrimental autonomic modulating properties that may contribute to ischaemia, arrhythmia and sudden deaths. The significance of some of these observations has yet to be fully explored.

1.8 Review of current drug therapy in chronic heart failure: The effects on potential surrogate markers for mortality

A significant proportion of the studies carried out in this thesis is focussed on the interaction between the components of the RAAS pathway and the autonomic nervous system such as the assessment of aldosterone blockade on heart rate variability (HRV) and baroreflex sensitivity (BRS). The effects on these autonomic markers have important clinical implications. Recent compelling evidence linking the autonomic nervous system and cardiac mortality including sudden death [Barron and Lesh 1996], suggests that these autonomic markers such as HRV, BRS and ventricular repolarisation characteristics eg. QT dispersion, are of prognostic value and may serve as potential “surrogate” markers for mortality in CHF.

Therefore to place into perspective the clinical relevance of the studies carried out in this thesis, I have in this section, reviewed the literature on the prognostic value of these surrogate markers and the impact of current drug therapy in CHF on them.

The impact of the different drugs on mortality and these surrogate markers are summarised in *Table 1.3*. The markers looked at include heart rate and its variability (HRV), baroreflex sensitivity, QT dispersion, arrhythmias, late potentials and neurohormones.

1.8.1 Surrogate Markers In Chronic Heart Failure:

HRV

HRV is a strong and independent predictor of mortality following acute myocardial infarction [Odemuyiwa *et al* 1994, Hartikainen *et al* 1996, Kleiger *et al* 1987, La Rovere *et al* 1998]. Although there is clear evidence that patients with chronic heart failure also exhibit a reduced HRV, the prognostic implications of the different spectral components and disease severity is more complex in heart failure, and there have been conflicting reports (see *Table 1.2*). With regards to its predictive value for mortality, preliminary results from the UK HEART study which followed 433 NYHA II-III patients over a mean of 458 days, have revealed that HRV is a powerful independent predictor of mortality [Nolan *et al* 1998].

BRS

Like HRV, baroreflex sensitivity (BRS) is an established parameter used to assess cardiac autonomic tone. Whereas HRV reflects tonic vagal activity at rest, BRS is predominantly a marker of activated vagal activity [Schwartz *et al* 1992, Hohnloser *et al* 1994]. The prognostic value of BRS was first demonstrated by La Rovere *et al* [1988] in post MI patients and has been confirmed by the recent ATRAMI study [La Rovere *et al* 1998]. Subsequent studies not only confirmed the predictive value of BRS but have shown that it is superior to HRV [La Rovere *et al* 1998, Farrell *et al* 1991, Farrell *et al* 1992]. The prognostic value of a depressed BRS in heart failure has been demonstrated by Osterziel *et al* [1995].

QT Dispersion

Since the pioneering work by Day *et al* [1990], numerous studies have evaluated the usefulness of QT dispersion as a marker of arrhythmic risk and sudden death in different clinical settings [Barr *et al* 1994, Priori *et al* 1994, Buja *et al* 1993, Potratz *et al* 1993, Perkiomaki *et al* 1995, Darbar *et al* 1996]. Barr *et al* [1994] and Brooksby *et al* [1996] recently found QT dispersion to be associated with sudden death and total cardiac mortality respectively, in patients with chronic heart failure. In contrast, Fei *et al* [1996] did not find any association. QT dispersion is very much at its infancy stage and there are still unresolved issues particularly relating to standardisation of the methodology used. As such, the predictive value of QT dispersion has been questioned by several investigators [Zareba *et al* 1994, Surawicz 1996].

Signal-averaged ECGs and Ambulatory Holter (24 hr ECGs)

In recent years the signal-averaged ECGs and ambulatory Holter have been used extensively to predict the risk of sustained ventricular tachyarrhythmias and sudden death. The prognostic value of signal-averaged ECGs for arrhythmic events including sudden death in post myocardial infarction patients is well established [McClements and Adgey 1993, Breithardt *et al* 1983]. However in heart failure the prognostic value of late potentials is uncertain. Whereas some studies described a high incidence of arrhythmias and sudden death in patients with an abnormal signal-averaged ECGs [Mancini *et al* 1993, Ohnishi *et al* 1990], others did not find any correlation [Middlekauff *et al* 1990, Silverman *et al* 1995]. Similarly the ambulatory electrocardiogram has been used with variable success in risk stratifying for sudden

death in patients with CHF [Szabo *et al* 1994, Olshausen *et al* 1988, Huang *et al* 1983, Wilson *et al* 1983, Holmes *et al* 1985].

Neurohormones

Several plasma neurohormones have also been found to be associated with increased mortality in CHF. Natriuretic peptides is of prognostic value in both congestive heart failure [Gottlieb *et al* 1989, Hall *et al* 1994, Swedberg *et al* 1990] and myocardial infarction [Hall *et al* 1994, Darbar *et al* 1996]. Plasma norepinephrine has also been shown to be an independent prognostic predictor for mortality in patients with symptomatic [Cohn *et al* 1984] and asymptomatic LV dysfunction [Benedict *et al* 1996]. The CONSENSUS I study found that in addition to catecholamines, increased levels of AngII, aldosterone and ANP were significantly correlated with mortality [Swedberg *et al* 1990]. Similarly the SAVE group had shown that other neurohormonal markers such as plasma renin activity and ANP provided significant predictive information about 1 year cardiovascular mortality [Rouleau *et al* 1994]. Endothelin 1 has also recently been shown to correlate negatively with 1 year survival [Pacher *et al* 1996].

General considerations with regard to surrogate markers:

Despite their predictive value for mortality, the above parameters cannot be accepted as surrogates to assess efficacy of drug therapy unless it can be shown that drug therapy which alters mortality can cause a corresponding change in the markers. Drug therapies that improve cardiac markers without any evidence of mortality benefits are useless and could in some cases be potentially dangerous. For example,

attempts to suppress arrhythmias in post MI patients with flecainide and d-sotalol in the CAST [Akiyama *et al* 1991] and SWORD [Waldo *et al* 1996] trials respectively have been associated with an increased mortality.

The potential of all the above markers as surrogates to assess efficacy of drug therapy will be evaluated in the next section.

1.8.2 Drug therapy in Chronic Heart Failure

Drugs That Have A Favourable Effect On Mortality

ACE Inhibitors

The mortality benefits of ACE inhibitors have been attributed to both neurohormonal suppression and vasodilatation [Cohn *et al* 1991]. Furthermore, it has been shown that the effect of ACE inhibitors on mortality reduction was greater in those with a high baseline circulating norepinephrine levels or an activated renin angiotensin system [Benedict *et al* 1995]. This reduction in mortality is paralleled by changes in various markers of the autonomic tone including a decreased heart rate [CONSENSUS-1 1987], increased heart rate variability [Flapan *et al* 1992, Binkley *et al* 1993, Kontopoulos *et al* 1996], increased baroreceptor sensitivity [Osterziel and Dietz 1996, DibnerDunlap *et al* 1996, Marakas *et al* 1995], decreased QT dispersion [Barr *et al* 1997] and a reduction in malignant ventricular arrhythmias [Cleland *et al* 1984, Webster *et al* 1985, Fletcher *et al* 1993]. In summary therefore, all potential surrogates are favourably altered by ACE inhibitors, which correspond with their favourable effect on mortality.

Hydralazine-Isosorbide Dinitrate

The vasodilator combination of hydralazine and isosorbide dinitrate has been shown to reduce mortality in the V-HeFT I study [Cohn *et al* 1986]. It has no impact on arrhythmic events in patients with left ventricular dysfunction and a history of inducible ventricular tachycardia [Bashir *et al* 1992]. However unlike ACE inhibitors, the hydralazine plus isosorbide dinitrate combination is associated with a small but significant rise in heart rate and an increased plasma norepinephrine concentration during the first year of follow-up [Cohn *et al* 1991]. However the impact on autonomic markers such as heart rate variability and baroreceptor sensitivity is not known. In summary, unlike the situation with ACE inhibitors, there is a discrepancy here between their favourable effects on mortality and their unfavourable effects on heart rate and neurohormones.

Beta-Blockers

The beneficial effects of beta-blockers on mortality reduction in CHF are now well established. Over the years there have been a number of small studies which demonstrated favourable but not statistically significant benefits with beta-blockers [Waagstein *et al* 1993, CIBIS Investigators 1994]. However recently Carvedilol [Packer *et al* 1996] a second generation beta-adrenergic antagonist with α_1 -blocking effects has been shown to reduce mortality by 65% when compared to placebo in CHF. Since then there has been two further large randomised trials published which also demonstrated mortality reduction with the beta-1 selective agents, metoprolol and bisoprolol [MERIT-HF Study Group 1999, CIBIS-II Investigators 1999].

In addition to heart rate reduction [Waagstein *et al* 1993, CIBIS Investigators 1994], beta-blockers have consistently improved heart rate variability [Kontopoulos *et al* 1996, Coumel *et al* 1991, Pousset *et al* 1996] and plasma neurohormones (eg.ANP, catecholamines) [Nemaniach *et al* 1990, Eichhorn *et al* 1991]. Beta blockers have also been reported to reduce ventricular tachyarrhythmias [Steinbeck *et al* 1992, Antz *et al* 1995]. The reports on the effects of beta-blockers on baroreceptor sensitivity are however conflicting [Parati *et al* 1983, Sanderson *et al* 1997, Lucini *et al* 1993]. With regard to QT dispersion beta-blockers have also been shown to reduce QT dispersion in the long QT syndrome [Priori *et al* 1994, Napolitano *et al* 1996]. Napolitano *et al* [1996] found that beta-blockers caused a greater reduction in those with higher baseline values. Sotalol also reduced QT dispersion in post-myocardial infarction patients [Day *et al* 1991]. Therefore as far as beta-blockers are concerned, there is good consensus between their effects on mortality and their effects on all surrogates.

Drugs That Have No Overall Impact On Mortality

Digoxin

Until recently the use of digoxin therapy in CHF and sinus rhythm has been controversial. Digoxin has been shown to reduce heart rate significantly in some studies [Brouwer *et al* 1995, Krum *et al* 1995] but not in others [Newton *et al* 1996]. Furthermore, it has also been shown to have favourable autonomic modulating properties; it restores HRV [Brouwer *et al* 1995, Krum *et al* 1995], improves baroreceptor sensitivity [Ferrari *et al* 1981] and reduces circulating catecholamines [Brouwer *et al* 1995, Ferguson 1992, van Veldhuisen *et al* 1993]. However despite

these promising signs digoxin had no impact on mortality in the DIG trial [1997] or on arrhythmic events in the DIMT study [van Veldhuisen *et al* 1993].

Calcium Channel Antagonists

The use of Ca-channel blockers in heart failure has been controversial because of their potentially negative inotropic activity and their ability to activate the neurohormonal system [Packer 1989]. However recent evidence have shown that the second generation dihydropyridines (eg.amlodipine or felodipine) possess more favourable neurohormonal effects [Devries and Dunselman 1995, Goldsmith 1995, Devries *et al* 1995]. They appear to have no adverse effects on plasma norepinephrine levels in both normal subjects [Goldsmith 1995] and heart failure patients [Devries and Dunselman 1995, Devries *et al* 1995]. The long term impact of these drugs on heart rate has not been reported from large trials. Although some dihydropyridines are known to cause an acute reflexogenic rise in heart rate, there are several small studies which show that the heart rate may subside in the long term [Lindqvist *et al* 1994]. The effects of calcium antagonists on HRV in CHF is not known but felodipine has been shown to have no significant effect on HRV in post-myocardial infarction(MI) patients [Bonaduce *et al* 1997]. Similarly, Cook *et al* [1991] could not show any effect with diltiazem in normal subjects. Only verapamil, which is not used in heart failure, has been shown to improve HRV in post MI patients [Ferrick *et al* 1990]. There is some data suggesting that calcium antagonists improve baroreflex sensitivity [Timmis *et al* 1984, Kassie and Armtorp 1987, Hirsch *et al* 1992] in heart failure. In mortality trials, amlodipine and felodipine appear overall to have a neutral effect [O'Connor *et al* 1995, Cohn *et al* 1995].

Amiodarone

Low dose amiodarone has been shown to reduce total mortality (including sudden death) by 28% over a 2 year period in the GESICA trial [Doval *et al* 1994]. However controversy has arisen as another trial CHF-STAT [Singh *et al* 1995] did not show any improvement in survival with amiodarone despite an improvement in LV ejection fraction. The discrepancy has not been fully explained but may be partially accounted for by differences in doses and characteristics of the patients used in the two studies. Results from the recent EMIAT and CAMIAT trials of post-MI patients (including those with LV dysfunction) have revealed a reduction in arrhythmic events although total mortality was not reduced. Nonetheless, amiodarone appears to have a favourable effect on potential surrogate markers; it has been shown to reduce heart rate significantly in both the GESICA and CHF-STAT studies. Furthermore it also improves heart rate variability [Zuanetti *et al* 1991, Woo *et al* 1994] and reduces QT dispersion [Cui *et al* 1994, Dritsas *et al* 1992].

Drugs With Adverse Effects On Mortality

Dopamine Receptor Agonists (Ibopamine)

Ibopamine is an active dopaminergic prodrug which works primarily as a vasodilator with some inotropic activity. It has no significant effects on heart rate [Brouwer *et al* 1995]. Furthermore it does not appear to have any significant proarrhythmic effects as documented by Holter monitoring and signal-averaged ECGs [van Veldhuisen *et al* 1991, van Veldhuisen *et al* 1993]. It has also been shown to modulate the autonomic tone favourably and to reduce circulating plasma

neurohormones [van Veldhuisen *et al* 1993, Metra *et al* 1995]. An improvement in HRV (although not statistically significant) has also been documented in a substudy of the DIMT study [Brouwer *et al* 1995]. Published long term survival data are unavailable at present but the multicentre trial PRIME II(second Prospective Randomised study of Ibopamine on Mortality and Efficacy) has been terminated due to adverse effects on mortality. Ibopamine would appear to be the most worrying example of disagreement between survival data and the results with potential surrogate markers, with the latter suggesting favourable effects which were far from reproduced in the mortality trial.

Flosequinan

Flosequinan is a novel vasodilator with inotropic properties, no longer in use because of its adverse effects on mortality [Packer *et al* 1993, Moe *et al* 1994]. It caused an increase in heart rate [Binkley *et al* 1994] and is associated with increased norepinephrine levels [Moe *et al* 1994]. However the worsened mortality is inconsistent with its effects on heart rate variability, where it appears to increase parasympathetic and decrease sympathetic tone respectively [Binkley *et al* 1994].

Positive Inotropic Agents (Milrinone)

Despite their beneficial effects on the haemodynamics of heart failure, phosphodiesterase inhibitors such as Milrinone have been shown to have adverse effects on mortality [Packer *et al* 1991]. They are known to activate the neuroendocrine systems especially the renin-angiotensin system [Smyth *et al* 1986] and predispose the myocardium to arrhythmias [Ferrick *et al* 1990]. The effects of

these inotropic agents on autonomic markers such as heart rate variability are unknown.

Anti-Arrhythmic Therapy

The use of antiarrhythmic therapy in chronic heart failure is limited. Apart from amiodarone, most antiarrhythmic drugs (especially Class I) have no significant effects on baseline heart rate but are associated with proarrhythmic effects, increased mortality and a deterioration in heart rate variability [Zuanetti *et al* 1991].

1.9 Scope of thesis and conclusions

Neurohormonal suppression in heart failure patients has a major impact on morbidity and mortality rates. In general, as one can clearly see from the previous section, drugs that have a favourable impact on mortality are those that cause neurohormonal suppression. In particular, the introduction of ACE inhibitors has revolutionised our management of heart failure patients in the 1980s. However, despite these benefits, CHF remains a lethal disease. It is now becoming clear that ACE inhibitors do not completely suppress the RAAS, and neurohormonal re-activation occurs with chronic ACE inhibition. The aldosterone “escape” may play a pivotal role in the regulation of the cardiac autonomic tone, the generation of malignant arrhythmias and ischaemia.

New therapeutic strategies to improve on the efficacy of ACE inhibitors on neurohormonal suppression of the RAAS include the introduction of specific Ang II antagonists, such as losartan, and the concomitant administration of aldosterone

antagonists, such as spironolactone, with ACE inhibitors. The impact of these new treatment strategies on mortality has recently been evaluated in large, randomised trials such as the ELITE II trial for losartan and the RALES trial (Randomised Aldactone Evaluation Study) [Pitt *et al* 1999] for spironolactone.

In this thesis, I have attempted to address some of the unresolved issues in this area of research. The thesis is divided into two main sections. The first are large clinical studies examining the consequences of aldosterone blockade in patients and the second are smaller mechanistic studies in normal man. Firstly, in two large clinical studies carried out in Chapters 3 and 4, the clinical significance of aldosterone escape was examined in further depth in both CHF and IHD patients respectively. In particular, the effects of aldosterone blockade on the diurnal profile of the cardiac autonomic tone, arrhythmic events and QT dispersion are described in order to try to understand the mechanisms underlying the mortality benefit seen in RALES. Furthermore, in Chapter 4, the intriguing possibility that aldosterone blockade has similar beneficial autonomic effects was explored in a cohort of patients with chronic stable angina.

The subsequent chapters consist of a series of mechanistic studies designed to explore some of the possible mechanisms in which aldosterone may contribute to autonomic dysfunction and increased dispersion of the QT intervals, a sensitive predictor of sudden death. In Chapters 5 and 6, the effects of the different components of the renin-angiotensin-aldosterone system on baroreceptor function were explored. Baroreceptor dysfunction occurs with worsening heart failure and is thought to predispose the myocardium to ventricular arrhythmias and sudden death. Although animal studies suggest that hyperaldosteronism may impair baroreceptor

function, this has not been previously documented in man. The paper in Chapter 5 describes for the first time, the effects of aldosterone on the baroreflex in man. The study in Chapter 6 on the other hand, describes the haemodynamic and baroreceptor responses of the Ang II antagonist, losartan, in comparison with an ACE inhibitor. The study was designed and carried out following the publication of the results of the ELITE I study [Pitt *et al* 1997] which suggested that losartan may well have an overall survival benefit (as a result of a reduction in sudden deaths), compared to ACE inhibitors. However the mortality benefits of losartan were not confirmed in the more recent ELITE II study. The study in chapter 6 may therefore provide us with valuable mechanistic insights as to why this may be the case.

Some of the potential mechanisms in which aldosterone may contribute to increased QT dispersion were explored in Chapter 7 where two separate studies were carried out. The direct effects of an intravenous infusion of aldosterone on QT dispersion were examined in one while the effects of increasing cardiac afterload (eg. vasoconstriction) and reflex vagal withdrawal on QT dispersion were described in the other.

In conclusion, CHF remains a complex clinical syndrome and the management of these patients continue to pose a difficult and challenging problem for clinicians. The therapeutic benefits of neurohormonal modulation in heart failure are now without doubt, as evident by the effects of ACE inhibitors, and more recently, of spironolactone and beta-blockers, on mortality. It is however disappointing as we enter the new millennium that despite all these advances, mortality rates remain high. The clinical significance of neurohormonal escape of aldosterone and Ang II despite ACE inhibitors is now being realised and opens up a whole new avenue for research

and drug development. The concept of aldosterone blockade given in addition to ACE inhibitors is an exciting yet simple therapeutic strategy. The studies carried out in this thesis provide a mechanistic insight and further enhance our understanding of the interaction between the RAAS, the cardiac autonomic tone and electrophysiology of the heart.



Figure 1.1: The Renin-Angiotensin-Aldosterone System

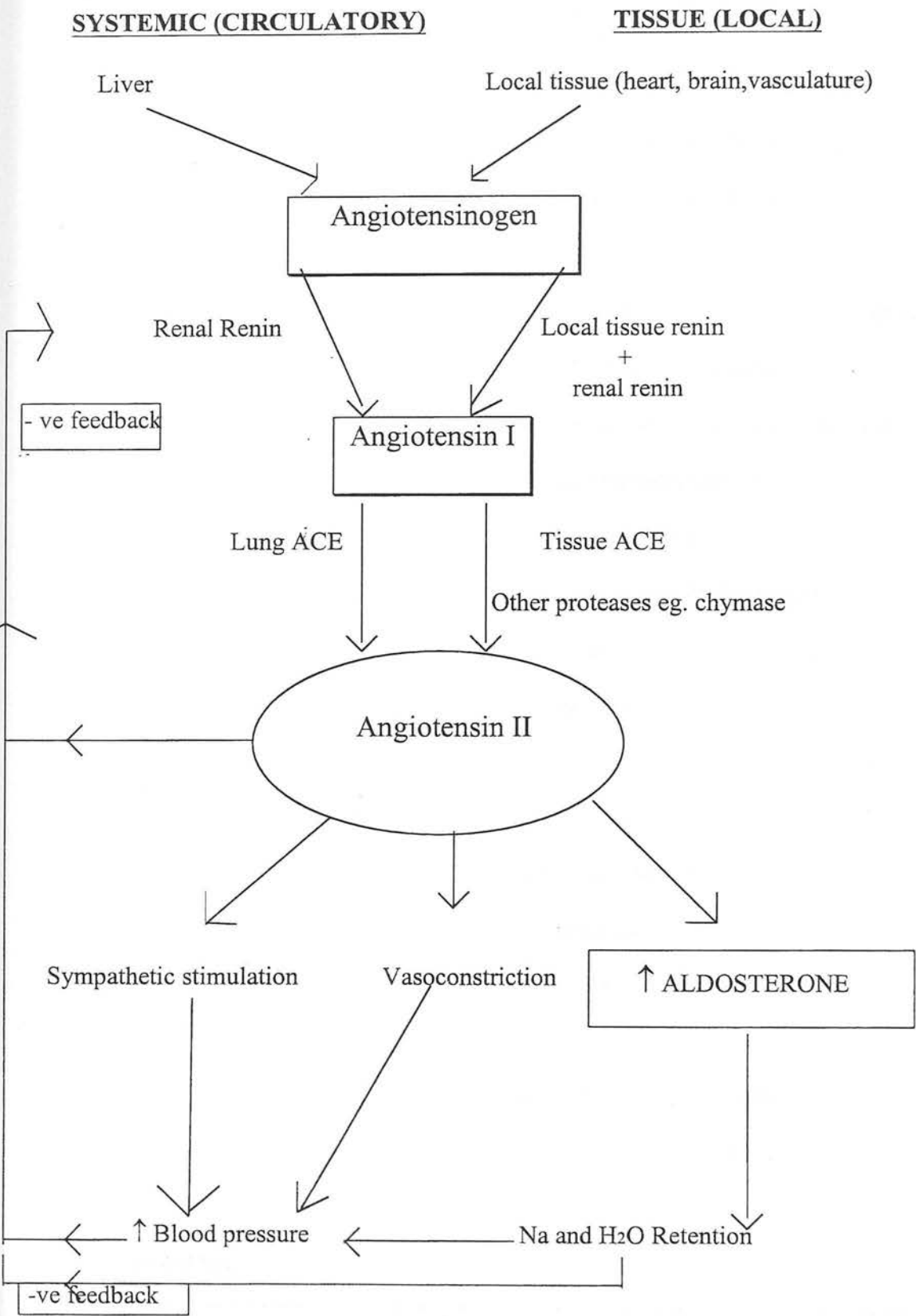


Table 1.1: Actions of Angiotensin II

<i>Site of action</i>	<i>Effect</i>
Kidney	<ul style="list-style-type: none">- vasoconstriction- decreases glomerular filtration rate- biphasic actions on tubular transport- redistribution of blood flow- antidiuretic
Vascular tissue	<ul style="list-style-type: none">- direct vascular smooth muscle constriction, hypertrophy and hyperplasia- potentiates neurogenic vasoconstriction- increases platelet adhesion- secretion of PAI-1- increases intimal lipid permeability? Atherogenic
Adrenal gland	<ul style="list-style-type: none">- increases Aldosterone secretion- increases medullary catecholamine release
Brain and Central Nervous System	<ul style="list-style-type: none">- sympathomimetic- parasympatholytic- increases thirst, salt intake- increases secretion of ADH, ACTH and oxytocin.
Heart	<ul style="list-style-type: none">- direct positive inotropic effect- cardiac myocyte hypertrophy- myocardial fibrosis
Gastrointestinal tract	<ul style="list-style-type: none">- stimulates fluid reabsorption

Table 1.2 Predictive Value Of Different Heart Rate Variability Parameters In Chronic Heart Failure:

STUDY	Patients number (n)	Duration of Follow up	Parameter used	Clinical Outcome / Correlation
Nolan et al 1998	442 NYHA II-III	458 \pm 156 days	SDNN, rMSSD, sNN50	SDNN<100ms was strongly predictive of death but rMSSD and sNN50 were similar among survivors and those who died.
Szabo et al 1996	173 NYHA III-IV	22 \pm 12 months	sNN50, SDNN	Both parameters significantly predictive of total cardiac death but not for arrhythmogenic / sudden death.
Woo et al 1996	57-Advanced Heart failure pts (mean LVEF 21%)	I year	SDANN, Poincare Plots (PLOTS)	Both parameters predictive of 1 year mortality (p<0.05).
Brouwer et al 1996	95 NYHA II-III	4 years	Both time and frequency domain parameters, Poincare Plots (PLOTS)	None of the time and frequency domain measures correlated significantly with survival. However abnormal PLOTS had independent prognostic value for sudden death and total cardiac mortality.
Binder et al 1992	61 LVEF<20%	17 months	Time and frequency domains	SDANN, total and low frequency bands correlated with total mortality.
Woo et al 1993	108	I year	SDANN, PLOTS	PLOT but not SDANN, predictive of sudden death.
Fei et al 1994	40 (mean LVEF 26%)	1 year	Frequency domain parameters	Frequency domain parameters not predictive of arrhythmic events or sudden death.

SUMMARY TABLE 1.3 The effects of drug therapy on mortality and potential surrogate markers in heart failure

DRUG	Mortality	HRV	Heart Rate	BRS	QT Dispersion	Holter Arrhythmias/ Signal-average ECGs	Neuro- hormones
ACE Inhibitors	G	G	G	G	G	G	G
β-Blockers	G	G	G	↓↑	G-n	G	G
Digoxin	0	G	0/G	G	?	0	G
Diuretics	?	?	0/G	?	?	↓↑	B
Ibopamine	B	G	0	?	?	0	G
Ca- antagonists	0	0-n	0/B	G	?	?	0
Hydralazine- Nitrate	G	?	B	?	?	0	B
Flosequinan	B	?G	B	?	?	?	B
Milrinone	B	?	?	?	?	B	B
Class I: Flecainide	B	B	0	?	?	↑↓	?
Class III: Amiodarone	0/G	G	G	?	G	G	?

Symbols:

G - good effect

B - bad effect

0 - neutral effect

? - not known

↓↑ - conflicting data

n - effect documented in non-CHF patient

CHAPTER TWO

SUBJECTS AND METHODOLOGY

2.1 *SUBJECTS*

Patient Subjects

All of the patient subjects entered into the studies described in this thesis were recruited from within Ninewells Hospital and Medical School in Dundee. The studies involving patients were conducted in accordance with the declaration of Helsinki as amended in Tokyo 1975, Venice 1983 and Hong Kong 1989. Details of patient selection are given in the appropriate chapters. The patients with chronic heart failure and chronic stable angina pectoris were recruited from the Heart Failure and Cardiology outpatient clinics respectively.

Healthy Volunteers

All volunteers were healthy normal subjects recruited by advertisements in the local hospital notice-boards at Ninewells Hospital. All had a normal medical assessment including physical examination, biochemical and haematological profile, urinalysis and electrocardiogram. Subjects who had a history of an adverse drug reaction, allergy or who required routine drug therapy were excluded. Subjects were also required to abstain from any self medication prior to and during the course of the studies. Sodium intake was not strictly controlled but each subject was asked to adhere to their usual diet throughout the study period and where possible, to maintain the same pattern of meals in the 48hrs preceding each study day. Subjects were also required to refrain from alcohol, caffeine and cigarettes for 24 hrs and to fast for 2hrs before each study session.

Consent

All subjects were provided with a study information sheet. Following a detailed explanation, subjects gave written informed consent and were advised of their right to withdraw from participation at any time.

Ethical approval

All studies were performed after approval by the local Tayside Committee on Medical Research Ethics.

2.2 METHODS

All the following procedures and analyses were performed by myself.

All intravenous infusions were administered through 18G intravenous cannulae placed in the antecubital veins. All infusions were given at the indicated rate by means of a constant infusion pump (IMED, San Diego, CA). Throughout each experiment, the electrocardiogram and heart rate were continuously monitored by an ECG oscilloscope (Hewlett Packard, USA) and blood pressure was recorded semi-automatically (Dinamap Vital signs monitor 1846, Critikon, Tampa, Florida, USA). All electrocardiograms [ECGs] (with simultaneous 12 lead acquisition for QT Dispersion analysis) were recorded using a Hewlett-Packard 4700A electrocardiograph.

The following sections describe the specific methodology and practical procedures performed in the studies constituting this thesis.

2.2.1. Arrhythmia / Ambulatory 24hr (Holter) Analysis

Twenty-four hour ambulatory electrocardiogram recordings were obtained for analysis in all subjects using a standard two-channel (four leads) *Tracker 2* analogue tape recorder (Reynolds Medical Limited, Hertford, UK), recording standard leads CM1 and CM5. The recorder provided continuous 24 hr recording of ECG at a tape speed of 1.47mm/sec, using a standard battery and low noise, magnetic tape Normal Bias Ferric Oxide cassette (TDK D90). It had a crystal generated timing track; the time of day was displayed on the Tracker and a time code recorded the real time accurately onto the tape. This also allowed for correction for recording and replay

speed errors (a feature essential for accurate measurement of heart rate variability). Subjects were also instructed to note their times of retiring to bed and rising to facilitate analysis.

Semiautomatic analysis of arrhythmias was performed using the Pathfinder 500 Series analyser system (Version 4.63 software, Reynolds Medical Limited, Hertford, UK). The trigger section of the analyser detected individual QRS complexes in the electrocardiogram, distinguishing them from P and T waves, muscle noise and artifacts using a continuously adaptive threshold derived from the noise components by independent filtering. The accuracy with which each QRS can be detected depended upon the quality of the recorded electrocardiograms, but it had been demonstrated that in routine ambulatory electrocardiograms, QRS detection errors occur at a rate of only twelve per 24 hrs [Neilson 1985]. Excessive noise sufficient to obscure QRS complexes automatically inhibited analysis until the noise subsides. The overall accuracy of QRS detection with this system was high, and the speed surveyor in the replay unit minimised inaccuracy due to speed variation [Neilson 1985, Bjerregaard 1980]. This is the only commercially available system whose performance had been evaluated by independent investigators [Stein *et al* 1994].

In addition, the recordings were also visually checked, and RR intervals and QRS configuration manually edited, to ensure correct arrhythmia recognition and classification. The classification criteria for arrhythmia analysis used by the Pathfinder 500 Series are shown in *Table 2.1*.

2.2.2. Heart rate variability (HRV) assessment

The measure of heart rate variability (HRV) describes variation of both

instantaneous heart rate and beat to beat RR interval changes. These oscillations in the interval between consecutive heart beats are mediated by fluctuations in the autonomic nervous system. Analysis of the ambulatory ECG recordings for HRV in the studies constituting this thesis was standardised and carried out according to the guidelines set by the joint Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology [1996]. HRV was assessed in both time and frequency domains. As above, the recordings were analysed using the Pathfinder 500 Series analyser. Segments of tape in which changes in RR interval duration arise due to occurrence of supraventricular or ventricular ectopic beats were excluded from analysis by the Pathfinder, which can identify non-sinus beats by their difference in timing and morphology when compared with sinus beats [Zuanetti *et al* 1991, Neilson 1975]. The Pathfinder analyser maintained a continuously updated example of each subject's normal QRS complex in its memory. Each detected QRS complex was compared with this and those with different morphology were labeled as ectopics and excluded from subsequent analysis [Neilson 1985]. The program also continuously maintained time thresholds corresponding to 63% and 175% of the prevailing normal RR interval, and rejected beats with intervals outside these limits. Interval calculations were recommenced from the third normal QRS complex down the line. Since the Pathfinder took no account of P wave morphology, and was thus unable to determine whether complexes showing minor degrees of prematurity were of sinus origin, the signal was closely monitored and edited by myself. For each QRS complex classified as sinus in origin, the Pathfinder generated an external pulse to signify the detection of a complex. The RR interval variability was then automatically analysed statistically as a function of time using parametric indices of measurement

(time domain analysis) by the Pathfinder with a validated software program (Reynolds Medical software version 4.63).

Frequency domain (power spectral) analysis of the RR interval variability on the other hand, was undertaken by Fast Fourier Transformation (FFT). Power spectral analysis provided the basic information of how power (or variance of the RR intervals) distributes as a function of frequency. The FFT is a complex mathematical device that allows this exploration of the periodic oscillations of heart rate at different frequencies by decomposing the signal into a series of sine waves of different amplitudes and spectral frequencies (see below). This process operated on 5 minute segments of data. The spectral measures were computed by FFT using a software programme (RR-Tools, Version 3.5, Reynolds Medical, Hertford, UK).

Recordings shorter than 18 hrs, or with less than 40% of the tape suitable for analysis were discarded. Separate analyses were undertaken for daytime (1000hrs - 2330hrs), nighttime (2330hr - 0630hrs) and dawn (0630hrs - 1000hrs). These times were prespecified before the studies began. The intra-observer variability for HRV analysis, as assessed by a second count on 10 tapes, was 5-6%.

Time domain measures of HRV

A variety of time-domain measures are available. A selection of the commonly used parameters in this thesis and their descriptions are listed in *Table 2.2*. The SDNN and HRV Triangular Index both provide estimates of the overall HRV [Task Force of the ESC 1996]. SDANN gives an estimate of the long-term components of HRV (ie. changes in heart rate due to cycles longer than 5 min) whereas both RMSSD and pNN50 estimate the short term components of HRV [Task Force of the

Frequency domain measures of HRV

In power spectral analysis, there are three main spectral components: very low frequency (VLF), low frequency (LF) and high frequency (HF) components (*Fig 2.1*). Measurements of VLF, LF and HF power components are made in absolute values of power (ms^2). LF and HF are also expressed in normalised units (n.u.), which represent the relative value of each power component in proportion to the total power minus the VLF component (see *Table 2.3*).

The physiological significance of the VLF component is uncertain and has not been clearly defined. The representation of the LF and HF in n.u. emphasises the controlled and balanced behaviour of the two branches of the autonomic nervous system. Vagal activity is the major contributor to the HF component. Some controversy exists with regards to the LF component - whereas some studies suggest that LF when expressed in n.u., is a quantitative marker of sympathetic modulation, other view LF as reflecting both sympathetic and vagal activity [Task Force 1996]. Consequently, the LF/HF ratio is considered by some to mirror sympathovagal balance [Task Force 1996]. In this thesis, both absolute power and normalised units are quoted to give a complete picture of the distribution of the spectral components.

2.2.3. ST segment analysis for cardiac ischaemia

ST segment analysis was carried out on the ambulatory electrocardiogram recordings using the Pathfinder 500 Series analyser. Standardised criteria for

ischaemia were used [Pepine *et al* 1987]. Significant ST depression for cardiac ischaemia was defined as $\geq 0.1\text{mV}$ (or 1 mm) planar or downsloping ST segment depression from baseline at 80ms after the J point lasting for more than 60sec. ST elevation was defined as the development of $\geq 0.1\text{mV}$ upward ST segment deviation at the J point. Each episode had to be separated by a return of the ST segment to baseline for at least 1minute to be counted as a discrete episode. The maximal depth of the ST segment depression during each episode was noted to allow the calculation of an index of ST segment depression (mm) X duration (min) as the “total ischaemic burden”.

Table 2.1: *Criteria for arrhythmia classification used by the Pathfinder
arrhythmia analyser*

Arrhythmia classification	Criteria
Pause	> 2.50 sec
Dropped beat	> 180% of RR Interval
Ventricular tachycardia (VT)	Minimum of 5 consecutive ventricular premature beats with a frequency of more than 100bt/min. Defined as non-sustained (NSVT) if last for < 30 sec and terminating spontaneously.
Salvo	Minimum of 4 consecutive ventricular premature beats
Bradycardia	Minimum of 4 consecutive beats at < 45 bt/min
SVT	Minimum of 5 consecutive beats at > 130 bt/min
Premature aberrant	< 66% of RR Interval
Premature normal	< 66% of RR Interval

Table 2.2 *Selected time-domain measures of HRV*

Statistical measure	Units	Description
SDNN	msec	Standard deviation of all NN intervals.
SDANN	msec	Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording.
RMSSD	msec	The square root of the mean of the sum of the squares of differences between adjacent NN intervals.
sNN50		Number of pairs of adjacent NN intervals differing by more than 50 msec in the 24 hr recording. The pNN50 is a derivative of this that expresses the sNN50 as a percentage of the total number of all NN intervals.
HRV triangular index		Baseline width of a triangle superimposed on the histogram of all RR intervals obtained in a 24 hr ECG. The position and configuration of the triangle is determined by the modal value and distribution of RR intervals in the frequency histogram, and excludes outliers.

Fig 2.1 *Power spectral density components: VLF (Very low frequency), LF (low frequency) and HF (High frequency)*

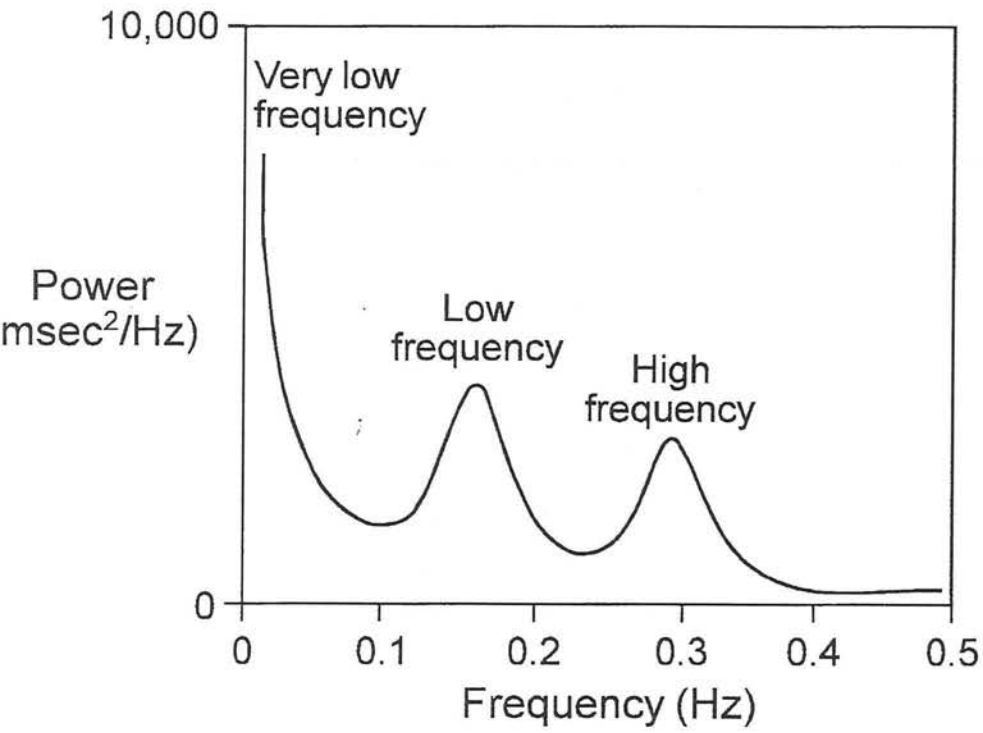


Table 2.3 *Selected frequency domain measures of HRV*

Measure	Units	Description	Frequency range
Total power	ms ²	Variance of all NN intervals	Approx. <0.4 Hz
VLF	ms ²	Power in the very low frequency range	< 0.04 Hz
LF	ms ²	Power in the low frequency range	0.04 - 0.15 Hz
HF	ms ²	Power in the high frequency range	0.15 - 0.4 Hz
LF (norm.)	n.u.	LF power in normalised units ie: LF/ (Total power-VLF) X 100	
HF (norm.)	n.u.	HF power in normalised units ie: HF/ (Total power-VLF) X 100	
LF/HF		Ratio LF/ HF	

2.2.4 ECG and QT Dispersion analysis

QT interval analysis was performed on electrocardiograms (with simultaneous 12-lead acquisition) recorded with a Hewlett-Packard 4700A electrocardiogram machine (Palo Alto, California). ECGs from the following subjects were excluded from analysis: those in atrial fibrillation or whose predominant rhythm was of nonsinus in origin, bundle branch block, paced complexes on the ECG or those on anti-arrhythmic medication. All QT intervals and dispersion (QTd) were analysed blindly by myself on a Calcomp digitizing tablet (Twyford, Berkshire, United Kingdom) using customised software (Medical Computing Unit, Ninewells Hospital). QT intervals were measured in all leads, if possible, on a surface 12-lead ECG (25mm/s speed). QT interval was taken from the onset of the QRS to the end of the wave (ie. return to the T/P baseline). If U waves were present, the QT interval was measured to the nadir of the curve between the T and U waves. Three consecutive cycles were usually measured for each lead. In all of the studies, QT intervals were corrected with Bazett's formula ($QT_c = QT/RR^{1/2}$). In addition to Bazett's formula, the QT intervals in the study described in Chapter 8 were also corrected using Morrison and Hodges linear correction formula [Hodges *et al* 1983].

QTd was calculated in ECGs in which ≥ 9 leads were measurable. QTd is defined as the difference between maximum and minimum QT intervals ($QT_d = QT_{max} - QT_{min}$). Corrected QT dispersion (QTcd) is defined as the difference between maximum and minimum QTc ($QT_{cd} = QT_{cmax} - QT_{cmin}$). The intra-observer variability (obtained from 20 set of ECGs that were repeatedly measured on 2 separate occasions) was 3% and 6-7% for measurements of QT intervals and QT dispersion, respectively.

2.2.5 Assessment of baroreflex sensitivity

The method most widely used for assessment of baroreflex responsiveness was devised by the Oxford group [Smyth *et al* 1969]. Traditionally, with this method, small bolus injections of the α -adrenoreceptor agonist phenylephrine are given intravenously. Phenylephrine, a vasopressor agent, increases blood pressure and causes a reflex bradycardia. Increases in arterial BP activate mechanosensitive nerve endings in the carotid sinus and aortic arch, which then send sensory information to the central nervous system via the glossopharyngeal and vagus nerves. This in turn, results in increased reflex vagal efferent outflow to the heart. A linear relationship exists between the systolic blood pressure and the change in cardiac cycle length; the slope of this relationship has been taken as a measure of baroreflex sensitivity (BRS) [Smyth *et al* 1969, La Rovere *et al* 1998, Sullebarger *et al* 1990].

However, there are practical limitations with this method. The Oxford bolus method requires beat to beat arterial pressure measurement. This would either involve an invasive approach or more usually, the use of a device called the FINAPRES (FINger ARterial PRESure), designed specifically for non-invasive recording of beat-to-beat blood pressure. Unfortunately, this form of non-invasive beat-to-beat analysis was not possible to do in our studies as the FINAPRES machines are no longer produced or sold in the United Kingdom.

Hence in the studies described in Chapters 5 and 6, we have opted instead, to administer phenylephrine by a graded infusion technique to measure baroreflex sensitivity. In addition our technique described below, has several other advantages. Firstly, infusions allowed us to monitor HR and BP changes at steady state non-invasively at each incremental dose, and hence avoiding the need for invasive beat-to-

beat intrarterial measurements as required by the bolus method (and hence, lessening risk and discomfort to the subjects).

Secondly, our readings were taken at steady state and more importantly still each of our readings were taken in triplicate, which should minimise random measurement error. Furthermore in our studies, we were more interested in *changes or differences* in baroreceptor sensitivities between different treatments rather than in using absolute levels of BRS to compare one population with another. BRS measurements by infusion method are not equivalent to the bolus method. Unlike the bolus method, infusions allow for baroreceptor “resetting” to occur to some degree, (and therefore reduce or dampen the change in baroreflex response). Although BRS measurements by infusion method are not equivalent to the bolus method, it has been shown to be reproducible in several studies [Sullebarger *et al* 1990, Ferguson *et al* 1985].

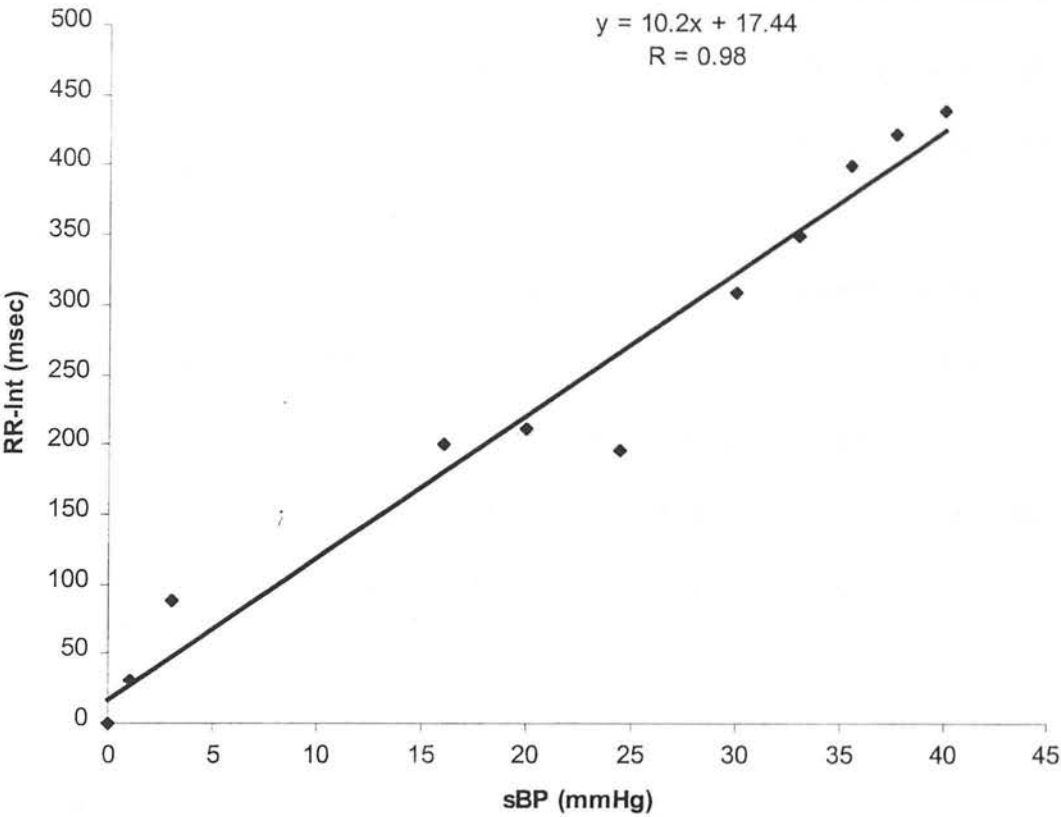
Infusion method of baroreflex sensitivity assessment:

The haemodynamic study was carried out after 30mins of supine bed rest. Intravenous PE was given by infusion into the right forearm. It was administered in stepwise 10 min infusions (0.2-3.6 ug/kg/min) by use of an infusion pump (IMED, San Diego, CA). The infusion was stopped when a 35-40 mmHg rise in systolic arterial pressure had been achieved. The average systolic BP and HR readings were obtained in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital signs monitor 1846, Critikon, Tampa, Florida, USA), and R-R interval obtained from continuous ECG recordings between 8 and 10 mins after each infusion dose.

The R-R intervals were plotted against the systolic blood pressure in a graph,

and a computerised curve fit was then carried out to establish a linear portion of the line of best fit. As described in other studies [La Rovere *et al* 1998, Sullebarger *et al* 1990, Marakas *et al* 1995], only regression lines that had a correlation coefficient of >0.8 were used; the slope of the linear portion of this relationship was taken as an index of baroreflex sensitivity (BRS). An example of BRS assessment in a normal volunteer is shown in *Fig 2.2*.

Fig 2.2: *Baroreflex sensitivity assessment in a normal volunteer*



BRS, measured as the slope of the linear regression line ($\Delta RR\ Int / \Delta sBP$), in this individual is 10.2 msec/mmHg.

2.2.6 Noradrenaline Kinetics Studies

The plasma concentration of noradrenaline is a composite of two concurrent processes: spillover of noradrenaline into plasma after release from sympathetic nerves, and subsequent removal of noradrenaline from the circulation. Thus a comprehensive assessment of sympathetic activity must take into account both processes. In this thesis, noradrenaline kinetics were determined by the well established technique of Esler *et al* [1979].

This radiotracer kinetic technique using infusions of [^3H]noradrenaline, can simultaneously assess noradrenaline spillover to the plasma and noradrenaline clearance. It requires the radiotracer to be infused until steady-state, and assumes that the infused [^3H]noradrenaline is not re-released to an appreciable degree, and that alumina extractable [^3H]metabolites do not accumulate during infusion.

1-(2,5,6- ^3H) noradrenaline infusion protocol:

All studies were performed in subjects in the supine position at rest. The tritiated noradrenaline infusion (NEN-DuPont, UK) was administered via an intravenous cannula inserted in the antecubital fossa. An initial loading dose was given as a bolus (12.5 uCi given iv over 2 minutes) followed by a constant infusion (0.7uCi/min/m²) for 60 minutes. This infusion regimen has been shown to reach steady-state and achieve constant plasma concentration of tritiated noradrenaline within 30 minutes [Esler *et al* 1979]. 20mls venous blood samples were taken for determination of resting plasma noradrenaline and tritiated noradrenaline levels after 40 and 50 minutes of the infusion (two samples were taken to ensure that steady state had been achieved).

Blood samples were taken in chilled lithium-heparin tubes, centrifuged at 3000rpm at 4 °C and the plasma separated and stored at -70 °C until assayed as a batch. Two 5mls samples of the infusate were also collected (one at the start prior to and the other after completion of infusion), stored and assayed as described for the blood samples, to allow determination of the actual rate of tritiated noradrenaline infusion.

Determination of noradrenaline kinetics:

Noradrenaline (NA) concentrations were measured by High Performance Liquid Chromatography (HPLC). All plasma samples were run on the same day in duplicate. After the addition of dihydroxybenzylamine used as an internal standard, catechols were extracted through adsorption on alumina. After elution from the alumina through the addition of 0.2 mol/L HClO₄, the catechols (NA and DHPG) were then separated and measured by HPLC. The HPLC effluent coinciding with the NA peak was collected and [³H]NA radioactivity levels determined by liquid scintillation spectroscopy. This allowed determination of the plasma [³H] NA concentration without interference from tritiated metabolites. The intrassay coefficient of variability was 6.1%.

From this data, NA spillover and clearance can be calculated using the following formulae:

$$\text{Whole body NA clearance} = \frac{[\text{^3H}] \text{NA infusion rate (dpm/min)}}{\text{Plasma } [\text{^3H}] \text{ NA concentration (dpm/ml)}}$$

$$\text{Whole body NA spillover} = \frac{[\text{^3H}] \text{ NA infusion rate (dpm/min)}}{\text{Specific radioactivity of plasma NA (dpm/pg)}}$$

2.3 MATERIALS AND ASSAYS

2.3.1 *Handling of blood samples prior to analysis*

Intravenous cannulae (18G) were placed into antecubital veins. Intermittent blood samples were taken from the indwelling cannula to cause the least disturbance to the resting subject.

Aliquots (5ml) were taken into chilled lithium heparin tubes for measurements of catecholamines (NA and DHPG) and aldosterone.

Aliquots (10ml) were taken into chilled glass tubes containing a solution of 0.05 M O-phenanthroline, 2 g/l neomycin, 0.125 M EDTA disodium salt and 2% ethanol, for measurement of Ang II.

Aliquots (20ml) were taken into chilled lithium heparin tubes for measurements of plasma [^3H] noradrenaline.

Aliquots (5ml) were taken into plain glass tubes for measurement of urea and electrolytes. The blood was allowed to clot at room temperature and serum separated and stored at $-20\text{ }^{\circ}\text{C}$ until assayed.

The samples taken for measurement of NA, DHPG, Ang II, aldosterone and plasma [^3H] noradrenaline were immediately centrifuged at $4\text{ }^{\circ}\text{C}$, separated and stored at $-70\text{ }^{\circ}\text{C}$ (NA, DHPG, Ang II and plasma [^3H] noradrenaline) or $-20\text{ }^{\circ}\text{C}$ (aldosterone) until assayed. All assays were done simultaneously in a batch.

2.3.2 *Analytical methods and biochemical assays*

Serum electrolytes

Plasma sodium, potassium, urea and creatinine were measured using a Chem Trak

system (Medical Analysis Systems, Camarillo, California) while plasma magnesium was measured by atomic absorption spectrophotometry (Pye Unicam atomic absorption spectrophotometer, Pye Unicam Ltd, Cambridge, UK). The intrassay coefficients of variability were 0.7%, 1.1%, 2.2%, 1.9% and 2.4% respectively.

Plasma catecholamines (Noradrenaline & Dihydroxyphenylglycol)

Plasma noradrenaline (NA) and plasma dihydroxyphenylglycol (DHPG) were measured by HPLC as described in the previous section on *Noradrenaline Kinetics*.

Specific radioactivity of plasma NA

See previous section on *Noradrenaline Kinetics*.

Plasma aldosterone

Plasma aldosterone was measured after plasma extraction, by a commercially available radioimmunoassay kit (Sorin Biomedica, Sallugia, Italy). The intra-assay coefficient of variation for this method in our laboratory was 7.8% and the inter-assay coefficient of variability was 9.6%.

Plasma Ang II

Plasma Ang II was measured after plasma extraction, by a commercially available radioimmunoassay kit (Nichols Institute Diagnostic BF, Nieuweweg, The Netherlands). The intra-assay coefficient of variation for this method in our laboratory was 4.0% and the inter-assay coefficient of variability was 9.3%.

2.3.3 List of Pharmacological Agents

d-Aldosterone: d-Aldosterone (Sigma Chemical Co Ltd, UK) was prepared as a solution (0.5mg/ml) for human administration to be diluted in 500ml Dextrose 5% for intravenous infusion by Tayside Pharmaceuticals, Dundee.

Phenylephrine: Phenylephrine hydrochloride 1% (Knoll Pharma,UK) was prepared as a solution (10 mg/ml) for human administration to be diluted in Dextrose 5% for intravenous infusion by Tayside Pharmaceuticals, Dundee.

Sodium nitroprusside: Sodium nitroprusside (non-proprietary) was prepared as a solution (10 mg/ml) for human administration to be diluted in Dextrose 5% for intravenous infusion.

1-(2,5,6- ³H) Noradrenaline: 1-(2,5,6- ³H) Noradrenaline (NEN-DuPont, UK) was prepared as a solution (100 uCi) diluted in 50ml N Saline containing Ascorbic Acid for human intravenous administration. The solution was prepared by the Medical Physics Department, Ninewells Hospital.

Spirolactone: Aldactone 50mg Tablets (Searle Ltd, UK).

Enalapril maleate: Innovace 20mg Tablets (Merck Sharp & Dohme Ltd, UK)

Frusemide: Lasix 40mg Tablets (Hoechst UK Ltd, UK)

Losartan potassium: Cozaar 50mg Tablets (Merck Sharp & Dohme Ltd, UK)

Note: All intravenous infusions were made up and/or diluted as indicated immediately prior to infusion.

2.4 STATISTICAL ANALYSIS

Unless otherwise indicated, all the data in this thesis were analysed using Statgraphics software package (STSC Software Publishing Group, MD, USA). Repeated measures analysis of variance was used to analyse the effect of treatments on the various HRV and QT dispersion indices measured. Subjects, treatment and dose were used as within factors for analysis. The effects of different treatment and placebo groups on other parameters were analysed using the paired Student t-test. Correlations between parameters were performed using standardised linear regression. A probability of $p < 0.05$ was considered as being of significance. Data and variables are expressed as arithmetic *Mean \pm Standard Deviation (SD)* unless otherwise stated. The specific statistical techniques in each study are detailed in the relevant chapters. Assistance on statistics was obtained from Mr. S Ogston from the Medical Statistics Department at Ninewells Hospital, Dundee.

CHAPTER THREE

ALDOSTERONE BLOCKADE AND AUTONOMIC REGULATION

IN CHRONIC HEART FAILURE

3.1 INTRODUCTION

In the RALES mortality trial, spironolactone therapy given in addition to ACE inhibitors, improved survival in chronic heart failure (CHF) but the mechanism of its benefit is not fully understood. Experimental evidence (as discussed in *Chapter 1*) suggests that aldosterone may have detrimental effects on the autonomic nervous system, especially during the morning hours when ACTH-induced aldosterone secretion is maximal.

Intriguingly, aldosterone secretion [Armbruster *et al* 1975, Davidson *et al* 1996] and the autonomic nervous system [Furlan *et al* 1990, Adamopoulos *et al* 1995] exhibit a diurnal variation, with both increasing in the early morning. Even more intriguingly, this early morning peak in aldosterone and sympathetic activity occurs at the same time of day as cardiac events peak [Muller *et al* 1985, Moser *et al* 1994, Raeder *et al* 1988]. Hence, the hypothesis arises that the dawn surge in aldosterone may unfavourably modulate the sympathovagal balance which then contributes to the high incidence of cardiac arrhythmias [Raeder *et al* 1988], ischaemia [Muller *et al* 1985] and sudden deaths [Moser *et al* 1994] seen particularly during the early hours of the day. In this study, I have sought to explore whether the dawn peak in aldosterone unfavourably alters the autonomic nervous system in CHF. A full diurnal assessment of the influence of aldosterone blockade on sympathovagal balance was carried out in CHF patients already established on ACE inhibitors. The circadian variation of autonomic function was assessed by heart rate variability analyses and norepinephrine kinetics studies. In addition the effects of aldosterone blockade on cardiac arrhythmias and on the diurnal variation of QT dispersion parameters on the 12 lead ECG were also investigated.

3.2 METHODS

Subjects

Twenty-eight patients with CHF (NYHA Class II to IV) and left ventricular ejection fraction (LVEF) $\leq 40\%$ were recruited from the heart failure clinic of our institute. All patients gave informed written consent and the protocol was approved by the local Tayside Ethics Committee on Medical Research. The baseline demographic characteristics of the patient population are listed in *Table 3.1*. All patients were on optimal therapy and had been treated with a diuretic and ACE inhibitor for at least 6 weeks before the study. LVEF was measured by either echocardiography or radionuclide ventriculography. Coronary artery disease was documented either by coronary angiography, positive exercise test or based on history of previous myocardial infarction. We excluded subjects whose predominant rhythm was of nonsinus origin (eg. atrial fibrillation), who had a bundle branch block or paced complexes on the ECG. Similarly, no patient recruited was taking anti-arrhythmic medication which might have altered the QT interval. All cardioactive drugs and their dosages were unchanged throughout the entire study (except for spironolactone or placebo).

Six patients had non-insulin dependent diabetes mellitus and three had hypertension. No patient had significant haematological abnormality, serum creatinine $>200\text{Umol/L}$, or abnormal liver function tests.

Protocol

All patients were recruited into a double-blind, randomised cross-over study. Each patient had been clinically stable for at least 3 months prior to recruitment. Baseline haemodynamic, biochemical and ECG measurements were made at the start of the study before each patient was randomised to receive spironolactone 50mg daily or placebo for a 1 month period for each treatment. Between each treatment phase, there was a washout period of 30 days. During each treatment, patients were reviewed weekly for clinical evaluation of tolerability and biochemical measurements of plasma urea and electrolytes.

At the end of each treatment phase, the patients were admitted to and studied at our research unit over a 24hr period. On arrival at 11am on each study day, all patients had two intravenous 18G indwelling cannulae inserted (one in each forearm), and a 24hr ambulatory Holter (Reynolds Tracker 2) monitor attached to their chest. After 30 mins of bedrest, baseline values of blood pressure and heart rate were determined in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, Florida).

A series of measurements were carried out during the 24hr spell. Repeated 12-ECGs and venous blood samples (10mls) for plasma norepinephrine were obtained at the following time points: 1500hr, 1700hr, 2300hr, 0300hr, 0600hr, 0700hr, 0800hr and 1100hr. Venous samples for plasma aldosterone (5mls) were also obtained at the 0600hrs and 1100hrs time points. The patients were asked to lie supine for 30 minutes prior to each sampling time-point. In addition, at 1600hr and 0700hr, the patients were administered an intravenous infusion of 1-(2,5,6-H) norepinephrine for assessment of norepinephrine kinetics (see *Chapter2*).

In between the study time-points, the patients were allowed to sit or move about the bedside. Excessive movements were kept to a minimum and they were instructed to record all their movements. Regular meals were provided for the 24hr period. Bedtime and mealtimes were standardised as follows: breakfast 0930hr, lunch 1300hr, dinner 1830hr and bedtime 2300 - 0700hr.

Ambulatory ECG monitoring

Twenty-four hour ambulatory electrocardiogram recordings were obtained for analysis in all subjects using a standard two-channel (four leads) *Tracker 2* analogue tape recorder (Reynolds Medical Limited, Hertford, UK), recording standard leads CM1 and CM5 as described in *Chapter 2*.

To facilitate analysis, subjects were instructed to note their times of retiring to bed and rising. Semiautomatic analysis of arrhythmias was performed using the Pathfinder 500 Series analyser system (Version 4.63 software, Reynolds Medical Limited, Hertford, UK). In addition, the recordings were also visually checked, and RR intervals and QRS configuration manually edited, to ensure correct arrhythmia recognition and classification.

Heart rate variability analysis

HRV was assessed in both time and frequency domains according to the guidelines set by the joint Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology [1996] and described in detail in *Chapter 2*. The following time-domain indices were evaluated from each 24 hr ECG recording: standard deviation of all RR intervals (SDNN), 24hr triangle

index, standard deviation of 5 min mean RR intervals (SDANN) and the root mean square of differences of successive RR intervals (rMSSD).

Frequency domain (power spectral) analysis of the RR interval variability on the other hand, was undertaken by Fast Fourier Transformation (FFT). This process operated on data of 5 min segments of each hour over the 24hr recording whilst the subjects were at rest. Time-periods where there were excessive movements (eg. subjects getting up to go to the toilet) were excluded from the analysis. Spectral plots were used to identify the low frequency (LF) component (0.03-0.14 Hz) and the high frequency (HF) component (0.18- 0.40 Hz). Indices are expressed in normalised units (n.u.) ie. the relative percentage compared to the total oscillatory power.

Recordings shorter than 18 hrs, or with less than 40% of the tape suitable for analysis were discarded. Separate analyses were undertaken for daytime (1000hrs - 2330hrs), nighttime (2330hr - 0630hrs) and dawn (0630hrs - 1000hrs). These times were prespecified before the study began, as the hypothesis was that the dawn period may be different from the rest.

QT interval and dispersion analysis

QT interval analysis was performed on electrocardiograms (with simultaneous 12-lead acquisition and all QT intervals and dispersion (QTd) were analysed blindly by myself on a Calcomp digitizing tablet (Twyford, Berkshire, United Kingdom) using customised software (Medical Computing Unit, Ninewells Hospital) as detailed in *Chapter 2*. The intra-observer variability (obtained from 20 set of ECGs that were repeatedly measured on 2 separate occasions) were 3% and 8-9% for measurements of QT intervals and QT dispersion, respectively.

Norepinephrine kinetics

All studies were performed in subjects in the supine position at rest according to the well established technique of Esler *et al* [1979] as described in *Chapter 2*. The tritiated norepinephrine infusion protocol was used twice per patient on each study day; the first infusion was commenced at 1600hr and the second repeated at 0700hr to allow us to assess norepinephrine spillover during the morning hours and evening hours. 20mls venous blood samples were taken in chilled lithium-heparin tubes for determination of resting plasma norepinephrine and tritiated norepinephrine levels after 40 and 50 minutes of the infusion (two samples were taken to ensure that steady state had been achieved).

Analysis of the blood samples and determination of the noradrenaline kinetics are detailed in *Chapter 2*.

Biochemical assays

See *Chapter 2*.

Statistical analysis

All the data were analysed using Statgraphics software package (STSC Software Publishing Group, MD, USA) as described in *Chapter 2*. Repeated measures ANOVA was carried out on the HRV and QT outcome measures. The within subject factors were treatment and time, and the between subject factor was order of treatment. A full factorial model was fitted to test the significance of these

factors. The effects of treatment on the other measures were analysed using the paired Student t-test.

3.3 RESULTS

Both spironolactone and placebo given in addition to ACE inhibitors were clinically well tolerated by all patients. There were no significant changes in baseline haemodynamic parameters such as resting heart rate and blood pressure. Plasma potassium, magnesium and urea were significantly increased with spironolactone compared to placebo after four weeks of treatment (see *Table 3.2*). Three of the patients had significant hyperkalemia (potassium > 5.5mmol/L) and elevation of plasma creatinine (>300umol/L) which necessitated the dose of spironolactone to be halved (25mg) in these individuals. Plasma aldosterone levels measured between 6 and 11am rose from 90 ± 39 pg/ml to 137 ± 60 pg/ml, and from 220 ± 80 pg/ml to 316 ± 108 pg/ml on the placebo and spironolactone study days, respectively.

The effects of spironolactone compared to placebo on the various autonomic parameters measured are displayed in *Tables 3-5*. Arrhythmic activity was unaffected by spironolactone therapy compared to placebo; there were no significant differences in the number of ventricular extrasystoles or non-sustained ventricular tachycardias (NSVT) between the two treatments.

Circadian pattern of heart rate variability

The indices of heart rate variability were significantly improved by spironolactone therapy compared to placebo. Although 24hr time domain measures

were not significantly affected by spironolactone therapy, there was a significant overall increase in the mean 24hr (averages of all the 5mins segments over the 24hr period) high frequency component (HF) and decrease in the low: high frequency ratio (LF/HF) throughout the 24hr period compared to placebo (*Table 3.3*). The effects of spironolactone on the HRV indices were greatest during the hours of awakening (0600-1000hr) (*Table 3.4, Fig 3.1-3.2*). During these hours, the standard deviation of RR intervals were significantly increased whereas both heart rate and the LF/HF ratio of the power spectral components were significantly reduced compared to placebo. The increase in the HF component by spironolactone was also highest during this time of day but the LF component was not significantly affected.

Circadian pattern of QT dispersion

QT interval dispersion indices were significantly higher during the day compared to the sleeping hours (*Fig 3.3*). All measured QT indices including QT_{cmax} and QT_d were significantly reduced by spironolactone compared to placebo (*Table 3.3 and Fig 3.4*). The reduction in QT_{cmax}, QT_d and QT_{cd} were greatest at the 6am time-point ($488 \pm 30\text{ms}$ to $470 \pm 33\text{ms}$, $p=0.04$; $68.0 \text{ ms} \pm 12.8 \text{ ms}$ to $55.0 \pm 9.5\text{ms}$, $p<0.001$ and $74.4 \pm 13.3 \text{ ms}$ to $62.0 \pm 9.5\text{ms}$, $p<0.001$ respectively).

Norepinephrine kinetics

The diurnal profile of plasma norepinephrine levels is displayed in *Fig 3.5*. There were no significant differences between the two treatments. Whole body NA clearance and spillover did not appear to be significantly affected by spironolactone therapy compared to placebo (*Table 3.5*).

Subgroup analyses

There were no significant differences in treatment effects between the NYHA Classes or between the NIDDM and the non-NIDDM subgroups (*Table 3.6*). Spironolactone improved both HRV and QT dispersion indices to the same degree in all subgroups.

Table 3.1: Baseline Demographic Characteristics of randomised patients.*Mean \pm SD (% in brackets)*

Characteristics	n=28
Age (yrs)	63 \pm 9
M / F	24 / 4
Weight (kg)	75 \pm 12
Actiology of CHF:	
Ischaemic	23 (82%)
Idiopathic dilated cardiomyopathy	3 (11%)
Unknown	2 (7%)
LVEF (%)	29 \pm 7
NYHA	
Class II	15 (54%)
Class III	13 (46%)
Frusemide	25 (89%)
ACE Inhibitor	28 (100%)
Ca antagonist	6 (21%)
Aspirin	21 (75%)
Nitrates	15 (54%)
Beta-blockers	2 (7%)
Digoxin	8 (29%)
History of Diabetes Mellitus	6 (21%)
History of Hypertension	3 (11%)

Table 3.2: Effect of Spironolactone and Placebo on resting haemodynamic and biochemical measures after four weeks of treatment .

Mean \pm SD. P <0.05 if statistically significant.

PARAMETER	PLACEBO	SPIRO	P VALUE
HR	72 \pm 13	70 \pm 12	NS
SBP	125 \pm 20	121 \pm 18	NS
K ⁺	3.9 \pm 0.4	4.3 \pm 0.4	0.01
Urea	7.6 \pm 3.5	8.8 \pm 4.1	0.01
Creatinine	120 \pm 31	137 \pm 37	NS
Mg ²⁺	0.82 \pm 0.05	0.85 \pm 0.06	0.05

Table 3.3 **Mean 24 hr Values:** *Effect of Spironolactone or Placebo on Arrhythmias, Heart Rate Variability and QT Dispersion. Mean \pm SD.*

	Parameter	Placebo	Spiro	p-value
<u>Time-domain</u>	RR Int	771 \pm 122	798 \pm 143	NS
<u>measures</u>	HRV Index	27 \pm 10	27 \pm 8	NS
	SDNN	91 \pm 32	92 \pm 26	NS
	SDANN	81 \pm 30	82 \pm 23	NS
	RMSSD	20 \pm 11	21 \pm 8	NS
<u>Frequency</u>	LF (n.u)	69.5 \pm 16.5	67.7 \pm 17.1	NS
<u>(Power Spectral)</u>	HF (n.u)	21.8 \pm 14.7	24.6 \pm 16.8	0.004
<u>measures</u>	LF/HF	4.98 \pm 3.54	4.40 \pm 3.10	0.002
<u>24hr arrhythmia</u>	Ventricular	2240 \pm 2125	2425 \pm 4555	NS
<u>analysis</u>	extrasystoles/24hr			
	NSVT(no.pats)	3	1	NS
<u>QT Interval and</u>	QTd	66.6 \pm 12.3	58.2 \pm 7.7	0.003
<u>Dispersion</u>	QTcd	73.2 \pm 13.4	65.3 \pm 7.6	0.0001
<u>variables</u>	QTmax	468 \pm 26	456 \pm 24	0.017
	QTcmax	480 \pm 34	467 \pm 30	0.001

Table 3.4: Diurnal Effects of Spironolactone and Placebo on HRVparameters: *Mean ± SD.*

Time (hr)	HRV parameter	Placebo	Spiro	p-value
1000 -2300 hr	RR (ms)	749 ± 130	780 ± 158	0.001
	SDRR (ms)	53 ± 25	56 ± 25	NS
	LF (n.u)	71 ± 14	70 ± 16	NS
	HF (n.u.)	21 ± 12	23 ± 15.5	0.031
	LF/HF	4.98 ± 3.35	4.65 ± 3.12	NS
2300-0600hr	RR (ms)	828 ± 143	845 ± 146	NS
	SDRR (ms)	53 ± 25	50.5 ± 23	NS
	LF (n.u)	65 ± 18	64 ± 19	NS
	HF (n.u.)	25 ± 19	27 ± 18	NS
	LF/HF	4.52 ± 3.56	4.13 ± 3.25	NS
0600-1000hr	RR (ms)	785 ± 154	821 ± 161	0.014
	SDRR (ms)	61 ± 30	71 ± 31	0.01
	LF (n.u)	71 ± 18	67 ± 16	NS
	HF (n.u.)	20 ± 14	27 ± 18	0.001
	LF/HF	5.62 ± 4.01	4.01 ± 2.76	0.002

Table 3.5: Effect of Spironolactone and Placebo on whole body Norepinephrine (NA) spillover and clearance. *Mean \pm SD.*

		Placebo	Spiro	P-value
AM (0700hrs)	NA <i>clearance (l/min)</i>	3.41 \pm 1.0	3.35 \pm 0.8	NS
	NA <i>spillover (ug/min)</i>	1.50 \pm 0.65	1.71 \pm 0.35	NS
PM (1600hrs)	NA <i>clearance</i>	3.32 \pm 0.8	3.0 \pm 0.7	NS
	NA <i>spillover</i>	1.75 \pm 0.82	1.83 \pm 0.63	NS

TABLE 3.6: Subgroup Analysis: Summary of main HRV effects in the NIDDM (n=6) and non-NIDDM subjects (n=22)

Mean ± SD. **p*<0.05 vs placebo.

	0600-1200hr		1200-1800hr		1800-2400hr		2400-0600hr	
	Placebo	Spiro	Placebo	Spiro	Placebo	Spiro	Placebo	Spiro
RR (ms)-non NIDDM	763 ± 144	796 ± 156*	749 ± 61	765 ± 95	762 ± 89	765 ± 93	811 ± 126	826± 123
-NIDDM	776 ± 108	800 ± 132	748 ± 55	759 ± 80	747 ± 77	733 ± 87	838 ± 101	850 ± 88
SDRR (ms)- non NIDDM	55 ± 30	64 ± 31*	53 ± 19	52 ± 22	52 ± 22	55 ± 23	48 ± 21	45 ± 20
- NIDDM	58 ± 28	65 ± 30	50± 18	48 ± 18	47 ± 16	50 ± 20	60 ± 15	66 ± 16
LF (n.u) - non NIDDM	75 ± 20	70 ± 13	76 ± 10	69± 11	67 ± 12	64 ± 10	64 ± 12	65 ± 18
-NIDDM	67 ± 15	64 ± 18	67 ± 10	72 ± 9	75 ± 8	80 ± 10	59 ± 9	63 ± 11
HF (n.u) - non NIDDM	20 ± 12	26 ± 18*	18 ± 10	23 ± 11	24 ± 8	20 ± 10	26 ± 10	29 ± 12
-NIDDM	23 ± 12	29 ± 15	22 ± 8	25 ± 10	20 ± 9	24 ± 8	22 ± 12	25 ± 13
LF/HF - non NIDDM	5.38 ± 3.5	4.51 ± 2.6*	4.80 ± 2.6	4.69 ± 2.7	5.12 ± 2.6	4.78 ± 2.5	4.54 ± 2.8	4.15 ± 2.9
-NIDDM	5.00 ± 3.3	4.33 ± 2.8	4.95 ± 2.5	5.21 ± 2.4	4.87 ± 2.8	5.04 ± 2.4	4.28 ± 3.0	4.44 ± 2.5

Figure Legends:

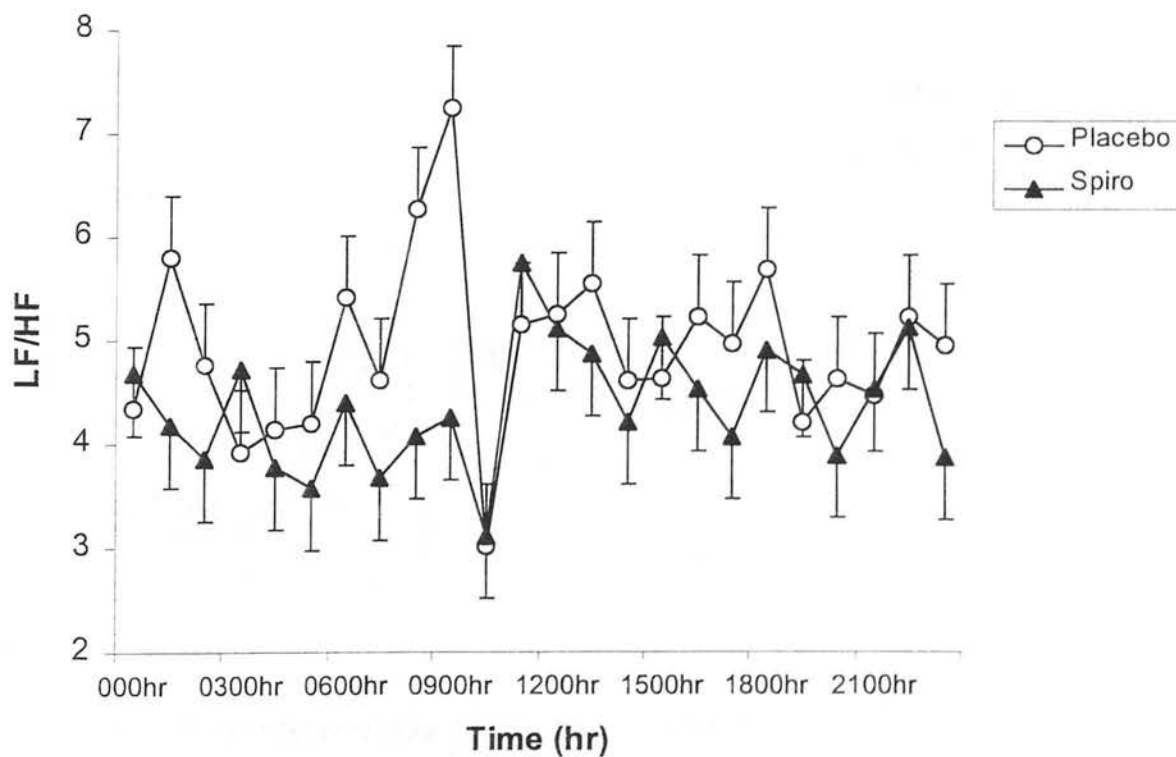
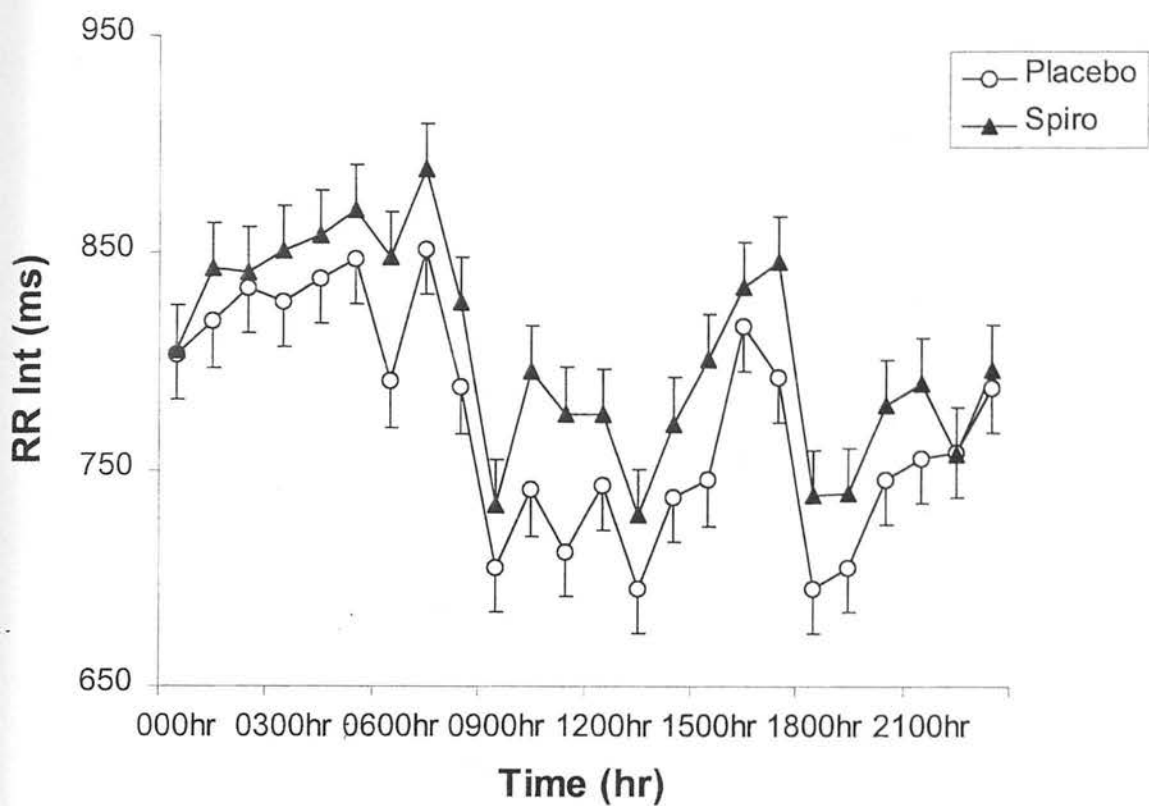
Figure 3.1: Diurnal variation of RR intervals (RR Int) [top] and ratio of low frequency to high frequency component (LF/HF) of heart rate variability [bottom]. Data expressed as Mean \pm SEM.

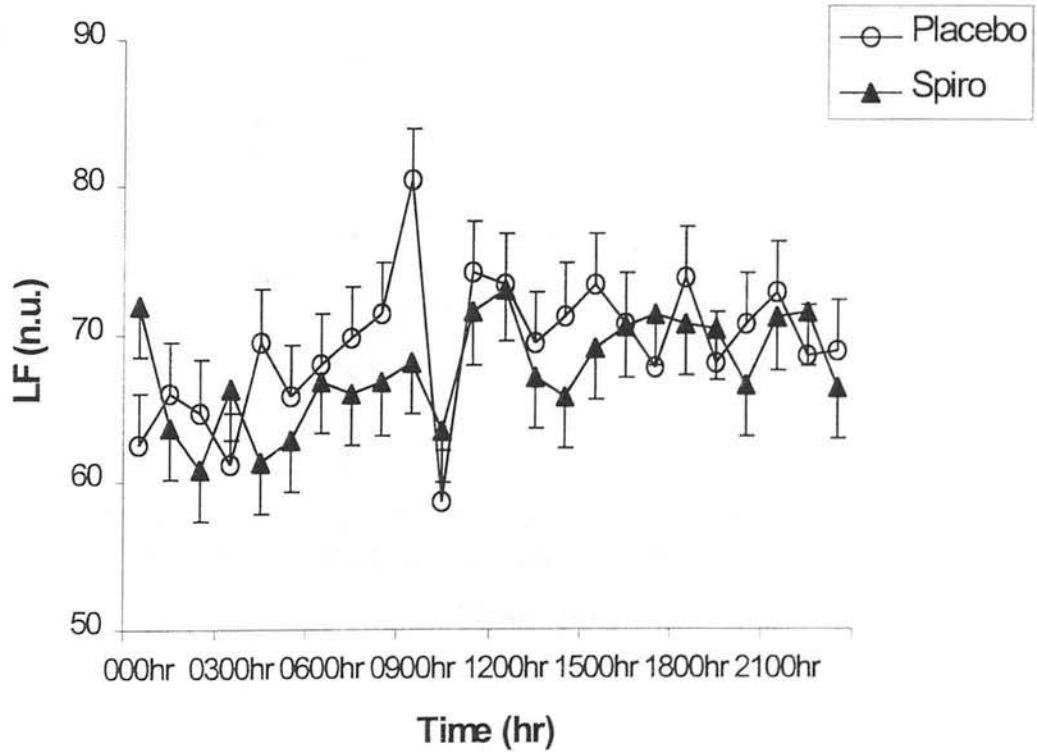
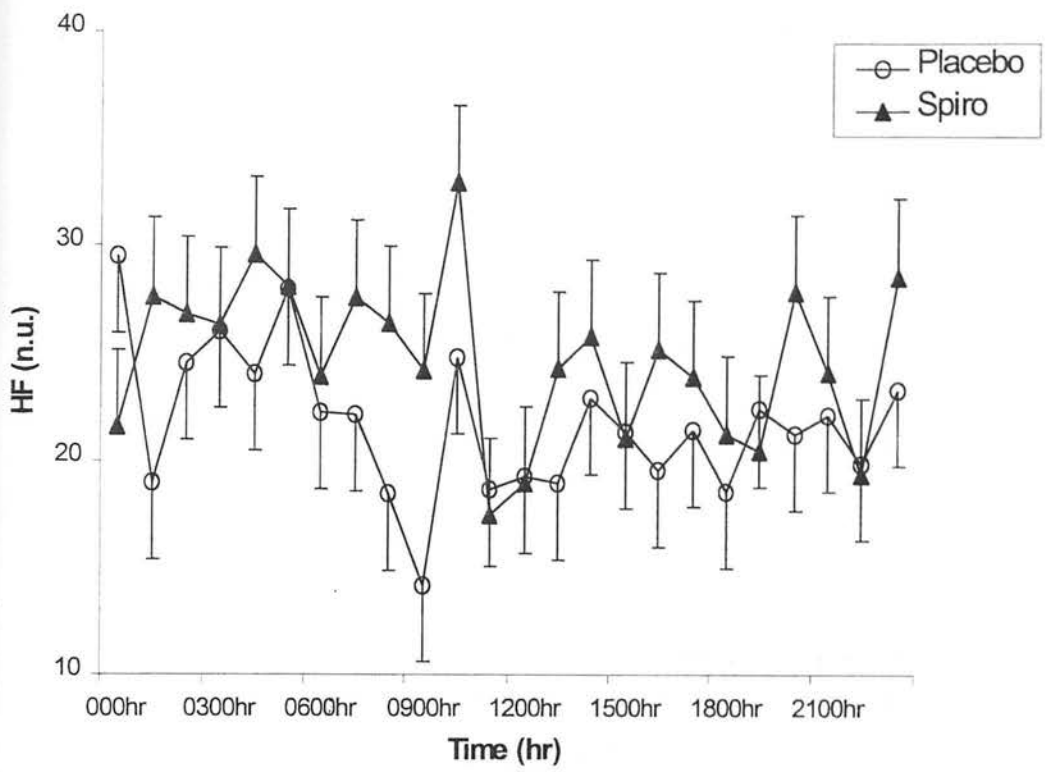
Figure 3.2: Diurnal variation of normalised high frequency component (HF) [top] and normalised low frequency component (LF) [bottom] of heart rate variability. Data expressed as Mean \pm SEM.

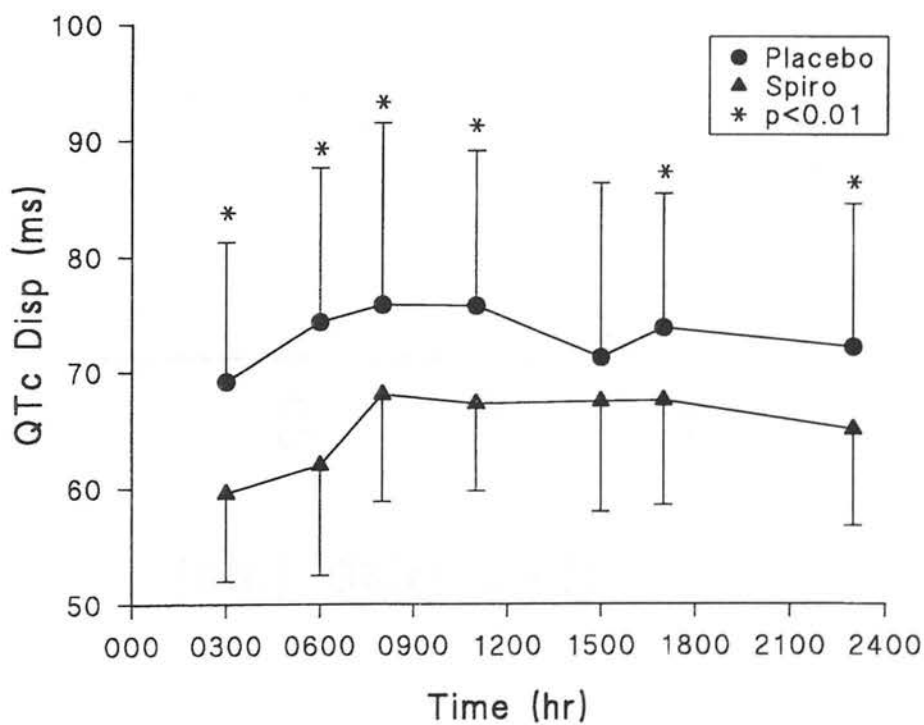
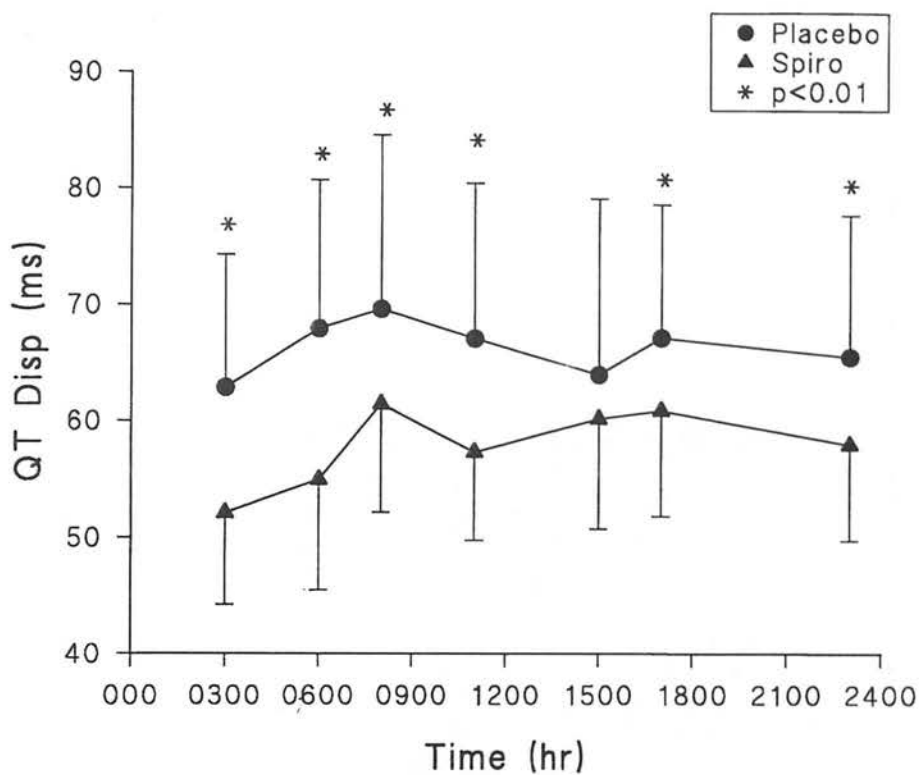
Figure 3.3: Diurnal variation of QT dispersion (QTd) [top] and Bazett's rate-corrected QT dispersion (QTc Disp) [bottom]. Data expressed as Mean \pm SEM.

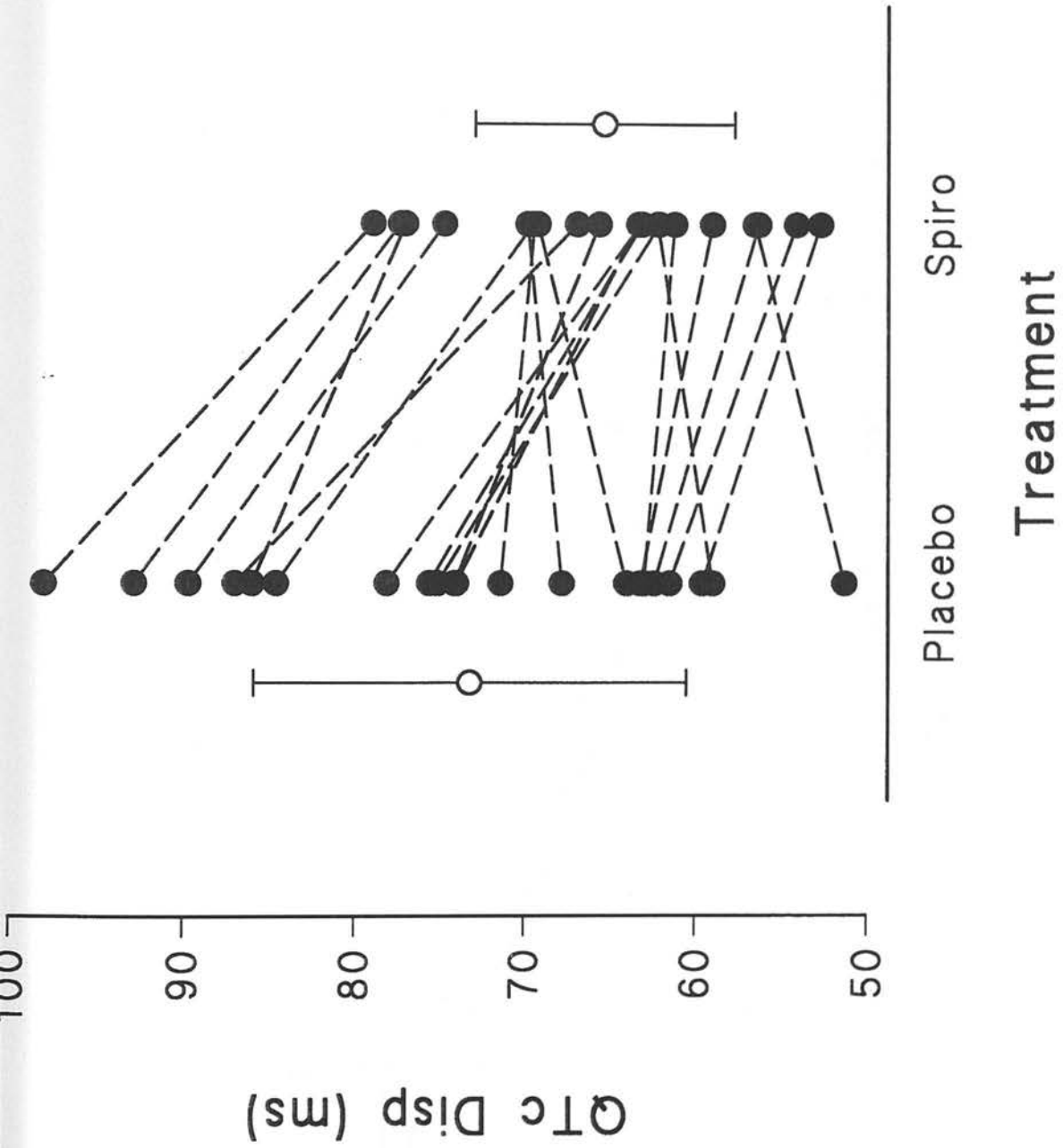
Figure 3.4: Individual mean 24hr QTc Dispersion (QTc Disp) data: The effects of Spironolactone and Placebo on QTc Disp.

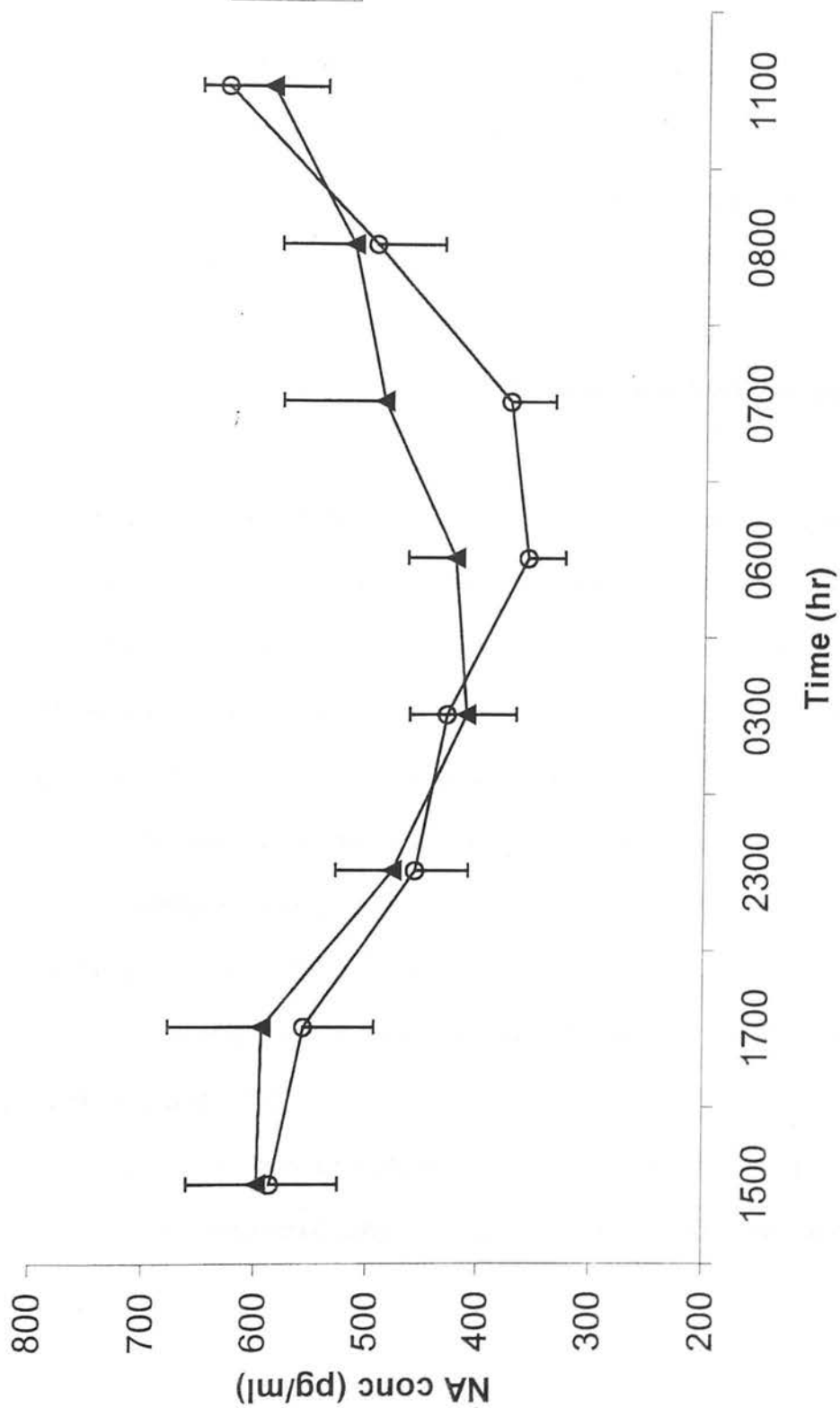
Figure 3.5: Diurnal profile of plasma norepinephrine levels (NA conc). Data expressed as Mean \pm SEM.











3.4 DISCUSSION

In the RALES study, the addition of spironolactone in CHF patients had been shown to confer a survival benefit over and above that of ACE inhibitors alone [Pitt *et al* 1999]. The results of this study provide mechanistic insights as to why this may be so.

In this study, the effects of spironolactone therapy in CHF patients already established on ACE inhibitors were examined. Consistent with previous findings [6,10,24,25], the addition of a small dose of spironolactone (50mg/daily) to maintenance treatment with a loop diuretic and ACE inhibitor in heart failure appear to be safe and well tolerated. After four weeks of treatment, spironolactone caused a small but significant rise in plasma potassium and magnesium levels, compared to placebo.

The effects of aldosterone blockade on various arrhythmic and autonomic surrogate markers were assessed in this study. Although ectopic activity was not significantly affected, spironolactone therapy was found to improve not only heart rate itself but also HRV as well as QT dispersion parameters. Perhaps the most intriguing aspect of our findings is that these effects appear to be maximal during the morning hours (6-10am), coinciding with the surge of aldosterone levels in man. Like cortisol, aldosterone secretion follows a diurnal variation with peak secretion during the morning hours [Armbruster *et al* 1975]. In CHF, it is well documented that this ACTH-induced dawn surge in aldosterone still occurs despite ACE inhibitor therapy [Davidson *et al* 1996].

As spironolactone was administered once daily, the question naturally arises whether the observed heightened early morning effects of spironolactone could also

be partly attributed to the pharmacokinetics of the drug itself. This is not likely as spironolactone has a very gradual and prolonged action with its activity persisting for more than 24hrs after a single dose, and steady state conditions are established within a few days [Gardiner *et al* 1989]. Furthermore the observed beneficial effects were observed from 6am onwards, which was well *before* the patients took the study medications (8-9am).

In this study, the magnitude of beneficial autonomic effects was similar across all subgroups of CHF patients. We could find no significant differences in HRV or QT dispersion changes between the NYHA subgroups, or between the NIDDM and the non-NIDDM subjects. It must however be pointed out that the study was *not* designed (and therefore was not statistically "powered") for subgroup analyses.

Aldosterone and diurnal sympathovagal modulation:

To date, there has been very limited data regarding the diurnal variation of the autonomic tone in CHF patients. Spectral analysis of 24hr HRV recordings have shown consistently that in normal subjects, LF and HF indices exhibit a circadian pattern and reciprocal fluctuations with higher values of LF in the daytime and of HF at night, corresponding with a sympathetic predominance during the day and vagal predominance during the night [Furlan *et al* 1990]. A major change in the autonomic profile occurs at approximately 6am, coinciding with the time of awakening, when a surge of sympathetic activity occurs rapidly and concomitantly with vagal withdrawal [Furlan *et al* 1990].

In CHF, this normal circadian variation of the autonomic tone is disrupted. Both time-domain and frequency-domain parameters of HRV are reduced

[Adamopoulos *et al* 1995, Casolo *et al* 1989, Casolo *et al* 1991, Panina *et al* 1995]. Reduced HRV has recently been shown to be a good predictor of cardiac death in CHF [Nolan *et al* 1998]. Data on the circadian variations of the spectral components are however limited and conflicting in CHF. In the present study, we observed a reduced but still preserved circadian pattern in the spectral components in CHF, a finding also reported by some [Adamopoulos *et al* 1995] but not others [Casolo *et al* 1989, Panina *et al* 1995].

This circadian pattern is further enhanced or preserved following spironolactone therapy. In particular, the HF spectral component was significantly increased whereas the LF/HF ratio was significantly reduced during the hours of awakening (6-10am). Interestingly, heart rate was also significantly reduced during this crucial time-period, a finding that was previously observed in another study [MacFadyen *et al* 1997]. These findings suggest that aldosterone blockade may have a favourable impact on the sympathovagal balance during these hours when aldosterone secretion is high. On the other hand, we found that the overall 24hr time domain measures of heart rate variability did not significantly differ between the two treatment groups. Although time domain methods are ideal for long-term recordings, they are not as robust as frequency domain methods, which are preferred for shorter term recordings [Task Force 1996]. By analysing short time segments (5mins) of each hour, the latter method better allowed us to assess the diurnal impact of aldosterone blockade at different time periods of the day. As aldosterone secretion occurs in a diurnal fashion, it is thus not surprising to find that the overall 24hr time domain HRV indices were not significantly altered, whereas between the 6-10am time period, the standard deviation of the RR intervals were significantly increased,

an observation which is consistent with the changes seen with the frequency domain indices.

How should one interpret these data? Vagal activity is thought to be the major contributor to the HF component [Task Force 1996]. Whereas some studies suggest that LF, when expressed as n.u., is a quantitative marker for sympathetic modulations, others view LF as reflecting both sympathetic and vagal activity [Task Force 1996]. Consequently, the LF/HF ratio is considered by some investigators to mirror sympathovagal balance [Task Force 1996]. Thus the favourable impact of spironolactone therapy on both the HF and LF/HF (but not LF) indices suggest that it works predominantly on the vagal limb of the autonomic tone. This is also consistent with the observation that spironolactone had no effect on norepinephrine kinetics. There is certainly a growing body of experimental evidence that aldosterone has detrimental effects on vagal activity. It has been shown to reduce baroreceptor discharge from the carotid sinus in experimental dog models [Wang *et al* 1992].

The role of aldosterone in sympathetic modulation is however, more controversial. Although there is some evidence in the literature to suggest that aldosterone may potentiate sympathetic activity [Barr *et al* 1995, MacFadyen *et al* 1997], we were unable to confirm this in our study. The diurnal plasma norepinephrine profiles were similar with both treatments; and neither whole body norepinephrine spillover nor clearance was significantly affected. These findings are not necessarily a contradiction to previous observations where cardiac norepinephrine uptake as assessed by MIBG scanning was increased by spironolactone therapy [Barr *et al* 1995]. The sympathetic nervous system is highly differentiated and the effects observed in the body as a whole may not be

representative of adrenergic drive specifically to the heart [Haskings *et al* 1986]. Newton *et al* [1996] made a similar observation with digoxin therapy in CHF patients where they found that digoxin administration reduced cardiac norepinephrine spillover but did not affect total body norepinephrine kinetics.

Aldosterone and QT Dispersion:

Another interesting observation in this study is the finding that spironolactone also reduced QT dispersion compared to placebo. The diurnal pattern of the QTc interval and dispersion in normal man is well established [Molnar *et al* 1997, Ishida *et al* 1997] but we believe that ours is the first study to show that QT dispersion occurs in a diurnal fashion in CHF patients, with daytime values being significantly higher than night time values.

Following spironolactone treatment, QTc_{max} and all indices of QT dispersion (QTd and QTcd) were significantly reduced at nearly all time-points compared to placebo (*Table 3.3, Fig 3.4*). This effect was greatest in the morning hours where there was a 19% reduction in QTd compared to placebo. Even more important is the fact that of the six patients who had a QTd>80msec on placebo, none of them had a QTd>80msec on spironolactone.

These observations may have important clinical implications. QTc interval prolongation has repeatedly been shown to be an independent risk factor for sudden cardiac death (SCD) [Schwartz and Wolf 1978, Algra *et al* 1991, Naas *et al* 1998]. Similarly, QTd has recently also been identified as a simple and easily measured predictor of SCD [Buja *et al* 1993, Barr *et al* 1994, Darbar *et al* 1996]. A QTd>80-85msec has been identified as a crucial threshold in predicting SCD [Naas *et al*

1998]. Although not fully understood, QTd is thought to be a measure of electrical inhomogeneity in the heart which decreases an individual threshold for malignant ventricular arrhythmias [Pye and Cobbe 1992]. There are a number of possible mechanisms in which aldosterone may contribute towards dispersion of the QT interval. As our heart rate variability data suggests, aldosterone may have detrimental autonomic modulating properties. Certainly decreased vagal activity and enhanced sympathetic activity has been linked with increased QT dispersion [Bonnar *et al* 1997]. The significance of these neural influences is further supported by the observations that the only drugs so far to have reduced QTd are those that modulate the autonomic tone, such as beta-blockers [Priori *et al* 1994, Day *et al* 1991] and ACE inhibitors [Barr *et al* 1997], which are well recognised to have vagomimetic effects [Flapan *et al* 1992, Osterziel and Dietz 1996].

Apart from its direct autonomic modulating effects, part of the beneficial effects of spironolactone may also be due to its diuretic effect (which may potentially reduce right atrial stretch and improve heart rate variability by mechanoelectric feedback [Horner *et al* 1996]). Certainly, in experimental models, an increase in cardiac afterload has been shown to be arrhythmogenic [Han *et al* 1990, Sideris *et al* 1995]. Therefore part of the reduction in QT dispersion seen with ACE inhibitors and spironolactone may be attributed to their ability to reduce cardiac afterload. These concepts are studied and discussed further in *Chapter 7*.

Furthermore, aldosterone-induced electrolyte depletion, in particular that of potassium and magnesium, may also contribute to dispersion of the QT intervals. Both hypokalemic and hypomagnesaemic states are arrhythmogenic, associated with prolongation of the QT intervals and increased likelihood of developing ventricular

arrhythmias such as torsade de pointes [Gettes 1976, Fisch 1973, Whang and Welt 1963]. Conversely, magnesium replacement therapy shortens the QT interval and has been shown to be a very effective anti-arrhythmic agent in terminating episodes of torsade de pointes [Tzivoni *et al* 1984]. The effects of magnesium on QT dispersion are at present unknown. Potassium, on the other hand, is one of the main determinants of the QT interval, as it is responsible for the outward repolarisation currents. Reduction of serum potassium, results in slower repolarisation and prolonged QT intervals [Nabauer *et al* 1993]. Recently, Choy *et al* [1997] observed that intravenous potassium infusion not only normalised QT prolongation but also reduces QT dispersion in CHF and in normal subjects pre-treated with quinidine. The relationship between potassium and QT dispersion may partly account for why spironolactone reduces QT dispersion.

There are therefore clearly a number of possible mechanisms by which spironolactone may reduce QTd, especially autonomic and potassium effects. As QTd has been shown to be a sensitive predictor of cardiac deaths, it would suggest that spironolactone may have properties which reduce malignant arrhythmias. Although arrhythmic events were not significantly influenced by the treatments in this study, it must be borne in mind that the sample size and therefore, the number of potentially lethal arrhythmic events such as non-sustained VTs recorded were very small.

Study limitations:

Firstly, the technical difficulty of obtaining a precise and reproducible determination of the QT interval, in particular the end of the T wave, is well

recognised. Care has been taken to use consistent criteria to define the end of the T wave and to exclude U waves. Although these methodological concerns are well appreciated, they were not a major issue for this study as we were looking at treatment induced *intraindividual* changes in QTd (rather than interindividual changes, which is the usual situation in previous case control studies).

Secondly, it must be pointed out that this study was carried out at a time when it was not routine practice to prescribe beta-blockers for CHF in the United Kingdom ie. before the beneficial effects of beta-blockers were established. Hence the majority of subjects were not taking beta-blockers at the time of the study. Although beta-blockers could alter the autonomic tone, many CHF patients cannot tolerate them. In any case, the main effect of beta-blocker therapy is on the sympathetic system whereas the main effect of spironolactone appears to be on the parasympathetic tone and QTd. Hence, the use of *both* treatments may actually be *synergistic* in reducing mortality, as suggested by the RALES subgroup analysis [Pitt *et al* 1999].

Conclusions:

Aldosterone blockade has a number of beneficial effects on the cardiovascular system in CHF. It reduces heart rate and improves both HRV and QT dispersion. The results of this study strongly suggest that its beneficial effects on the autonomic nervous system appear to be primarily parasympathomimetic in nature with minimal or little influence on sympathetic activity. The clinical significance of vagal tone modulation has been highlighted recently in a large prospective study

where reduced baroreflex sensitivity was found to correlate strongly with increased cardiac mortality [La Rovere *et al* 1998].

Hence the finding that spironolactone alters vagal tone favourably could potentially have important clinical implications. The effects of aldosterone on the parasympathetic tone are therefore, examined in further depths elsewhere in this thesis. In particular, the influence of aldosterone on the baroreflex is explored in a separate study in *Chapter 5*.

How spironolactone reduces QT dispersion is not fully understood. Again, this may also be another clinically important finding. As discussed above, there are a number of potential mechanisms, which may account for the reduction in QT dispersion by aldosterone blockade. It is unfortunately beyond the scope of the present study to tease out the different mechanisms. Some of these potential mechanisms are explored further in *Chapter 7*.

In summary, it would appear that the beneficial effects of aldosterone blockade are greatest during the morning hours, coinciding with the circadian pattern of cardiovascular events such as sudden death and myocardial infarctions. Aldosterone secretion under the influence of ACTH is known to peak at this time of the day, and therefore it is not surprising that the autonomic effects of spironolactone are most prominent then. By exerting its maximal effects during the morning hours (coinciding with the peak of cardiovascular events), it may particularly reduce the number of such events in the dawn period of the day. Unfortunately, the RALES study has not recorded accurate timings of death within the day to see if spironolactone reduces deaths more at dawn than at other times, which means that

the postulated effect of spironolactone on early morning deaths must remain for now an intriguing hypothesis.

The intriguing findings - the reduction in heart rate and favourable sympathovagal modulation at dawn - also raise the possibility that spironolactone may have anti-ischaemic properties during these crucial hours. In the following chapter, I have attempted to address the issue of whether the spironolactone-induced reduction in heart rate at dawn would translate into a meaningful reduction in ischaemic events in a study of patients with angina pectoris, a cohort who is particularly at risk of dawn ischaemic events.

CHAPTER FOUR

ALDOSTERONE BLOCKADE AND AUTONOMIC REGULATION

IN CHRONIC STABLE ANGINA PECTORIS

4.1 INTRODUCTION

It was intriguingly observed in the previous study of CHF patients that aldosterone blockade improved heart rate variability and reduced the dawn (6-10am) increase in heart rate. This may be a clinically important observation as a dawn increase in heart rate could be pro-ischaemic and contribute to the well known dawn increase in sudden deaths. Spironolactone may therefore have early morning anti-ischaemic effects by way of reducing heart rate during these crucial hours.

In addition to an increase in early morning heart rate, the detrimental effect of aldosterone on the sympathovagal tone has also been linked with an increase in coronary artery tone [Weber and Purdy 1982, Hatakeyama *et al* 1994].

In addition to these autonomic modulating properties, aldosterone is also known to increase urinary potassium and magnesium electrolytes loss [Rahman *et al* 1992], and magnesium depletion could cause coronary vasoconstriction [Turlapaty and Altuna 1989]. It was also recently observed that aldosterone blockade dramatically improved endothelial dysfunction and increased vascular nitric oxide bioactivity in CHF patients [Farquharson and Struthers 2000]. Finally, aldosterone itself may be implicated in the pathogenesis of atherosclerosis. Furthermore monocytes which are implicated in the development of atherosclerosis, appear to have functionally active aldosterone receptors [Wehling *et al* 1987].

Hence there is a growing body of evidence to suggest that circulating aldosterone could have detrimental autonomic, pro-ischaemic and arrhythmogenic effects. However the data above all refer to CHF patients who tend to have neuro-endocrine activation. The question naturally arises whether spironolactone would demonstrate similar effects in patients with stable angina pectoris, especially since

these patients are at risk of dawn ischaemic events. Therefore like the previous study, we conducted a full diurnal assessment of the influence of aldosterone blockade on sympathovagal balance to see if spironolactone altered autonomic tone in the vulnerable early morning period in patients with stable angina pectoris. The circadian variation of autonomic function was assessed comprehensively by both time and frequency-domain (power spectral) heart rate variability analyses and norepinephrine kinetics studies. The study was also carried out to see if the previously observed spironolactone-induced bradycardia at dawn would lead to a reduction in ischaemic events.

Like the previous study, the effects of aldosterone blockade on cardiac arrhythmias and the diurnal variation of QT dispersion parameters on the 12 lead ECG were also examined.

4.2 METHODS

Subjects

Twenty-seven patients with chronic stable angina [Mean age (SD) 64 (9)] were recruited from the Cardiology out-patient clinic of our institute. All patients gave informed written consent and the protocol was approved by the local Tayside Ethics Committee on Medical Research. The baseline demographic characteristics of the patient population are listed in *Table 4.1*. All patients were clinically stable and had been established on standard anti-anginal therapy for least 6 weeks before the study. Coronary artery disease was documented either by coronary angiography (17

subjects), positive exercise test (7 subjects) or radionuclide thallium scanning (3 subjects). All subjects recruited had normal or near normal left ventricular ejection fraction. Subjects with ejection fraction <35% or who were in NYHA Class >II were excluded. Similarly we excluded subjects whose predominant rhythm was of nonsinus origin (eg. atrial fibrillation), who had a bundle branch block or paced complexes on the ECG. Similarly, no patient recruited was taking anti-arrhythmic medication which might have altered the QT interval. All cardioactive drugs and their dosages were unchanged throughout the entire study (except for spironolactone or placebo). The subjects were on regular anti-anginal therapy including beta-blockers (48%). Sixteen subjects (59%) were taking ACE inhibitors regularly.

Two patients had non-insulin dependent diabetes mellitus and three had hypertension. No patient had significant haematological abnormality, serum creatinine >200Umol/L, or abnormal liver function tests.

Protocol

All patients were recruited into a double-blind, randomised cross-over study. Each patient had been clinically stable for at least 3 months prior to recruitment. Baseline haemodynamic, biochemical and ECG measurements were made at the start of the study before each patient was randomised to receive spironolactone 50mg daily or placebo for a 1 month period for each treatment. Between each treatment phase, there was a washout period of 30 days. During each treatment, patients were reviewed weekly for clinical evaluation of tolerability and biochemical measurements of plasma urea and electrolytes.

At the end of each treatment phase, the patients were admitted to and studied at our research unit over a 24hr period. The protocol is similar to the CHF study described in the previous chapter. On arrival at 11am on each study day, all patients had two intravenous 18G indwelling cannulae inserted (one in each forearm), and a 24hr ambulatory Holter (Reynolds Tracker 2) monitor attached to their chest. After 30 mins of bedrest, baseline values of blood pressure and heart rate were determined in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, Florida).

A series of measurements were carried out during the 24hr spell. Repeated 12-ECGs and venous blood samples (10mls) for plasma norepinephrine were obtained at the following time points: 1500hr, 1700hr, 2300hr, 0300hr, 0600hr, 0700hr, 0800hr and 1100hr. Venous samples for plasma aldosterone (5mls) were also obtained at the 0600hrs and 1100hrs time points. The patients were asked to lie supine for 30 minutes prior to each sampling time-point. In addition, at 1600hr and 0700hr, the patients were administered an intravenous infusion of 1-(2,5,6-H) norepinephrine for assessment of norepinephrine kinetics (see below).

In between the study time-points, the patients were allowed to sit or move about the bedside. Excessive movements were kept to a minimum and they were instructed to record all their movements. Regular meals were provided for the 24hr period. Bedtime and mealtimes were standardised as follows: breakfast 0930hr, lunch 1300hr, dinner 1830hr and bedtime 2300 - 0700hr.

Ambulatory ECG monitoring

Twenty-four hour ambulatory electrocardiogram recordings were obtained for analysis in all subjects using a standard two-channel (four leads) *Tracker 2* analogue tape recorder (Reynolds Medical Limited, Hertford, UK), recording standard leads CM1 and CM5 as described in *Chapter 2*.

To facilitate analysis, as in the previous study, subjects were instructed to note their times of retiring to bed and rising. Semiautomatic analysis of arrhythmias was performed using the Pathfinder 500 Series analyser system (Version 4.63 software, Reynolds Medical Limited, Hertford, UK). In addition, the recordings were also visually checked, and RR intervals and QRS configuration manually edited, to ensure correct arrhythmia recognition and classification.

Heart Rate Variability analysis

HRV was assessed in both time and frequency domains according to standardised guidelines as described in *Chapter 2*. The following time-domain indices were evaluated from each 24 hr ECG recording: standard deviation of all RR intervals (SDNN), 24hr triangle index, standard deviation of 5 min mean RR intervals (SDANN) and the root mean square of differences of successive RR intervals (rMSSD).

Frequency domain (power spectral) analysis of the RR interval was undertaken by Fast Fourier Transformation (FFT). This process operated on data of 5 min segments of each hour over the 24hr recording whilst the subjects were at rest. Time-periods where there were excessive movements (eg. subjects getting up to go to the toilet) were excluded from the analysis. Spectral plots were used to identify the

low frequency (LF) component (0.03-0.14 Hz) and the high frequency (HF) component (0.18- 0.40 Hz).

Recordings shorter than 18 hrs, or with less than 40% of the tape suitable for analysis were discarded. Separate analyses were undertaken for daytime (1000hrs - 2330hrs), nighttime (2330hr - 0630hrs) and dawn (0630hrs - 1000hrs). These times were prespecified before the study began, as the hypothesis was that the dawn period might be different from the rest. All analyses were carried out blindly by myself. The intra-observer variability for HRV analysis, as assessed by a second count on 10 tapes, was 6-7%.

ST Segment analysis

ST segment analysis was carried out on the ambulatory electrocardiogram recordings using the Pathfinder 500 Series analyser, and standardised criteria for ischaemia were used, as detailed in *Chapter 2*.

QT interval and dispersion analysis

QT interval analysis was performed on electrocardiograms (with simultaneous 12-lead acquisition and all QT intervals and dispersion (QTd) were analysed blindly by myself on a Calcomp digitizing tablet (Twyford, Berkshire, United Kingdom) using customised software (Medical Computing Unit, Ninewells Hospital) as detailed in *Chapter 2*. The intra-observer variability (obtained from 20 set of ECGs that were repeatedly measured on 2 separate occasions) were 3% and 8-9% for measurements of QT intervals and QT dispersion, respectively.

Norepinephrine kinetics

All studies were performed in subjects in the supine position at rest according to the well-established technique of Esler *et al* [1979] as described in *Chapter 2*. The tritiated norepinephrine infusion protocol was used twice per patient on each study day; the first infusion was commenced at 1600hr and the second repeated at 0700hr to allow us to assess norepinephrine spillover during the morning hours and evening hours. 20mls venous blood samples were taken in chilled lithium-heparin tubes for determination of resting plasma norepinephrine and tritiated norepinephrine levels after 40 and 50 minutes of the infusion (two samples were taken to ensure that steady state had been achieved).

Analysis of the blood samples and determination of the noradrenaline kinetics are detailed in *Chapter 2*.

Biochemical assays

See *Chapter 2*.

Statistical analysis

All the data were analysed using Statgraphics software package (STSC Software Publishing Group, MD, USA) as described in *Chapter 2*. Repeated measures ANOVA was carried out on the HRV and QT outcome measures. The within subject factors were treatment and time, and the between subject factor was order of treatment. A full factorial model was fitted to test the significance of these factors. The effects of treatment on the other measures were analysed using the paired Student t-test.

Using variability data from Adamopoulos *et al* [1992], the study should have 90% power to detect a 10% change in high frequency (HF) heart rate variability and SD of RR intervals (SDNN) at $p < 0.05$, and have 80% power to detect a 15% change in plasma noradrenaline at $p < 0.05$. Similarly, based on previous data [Schuang and Pepine 1977, Deanfield *et al* 1983] the study should also have 90% power at $p < 0.05$ to detect an 18% reduction in duration of each ST depression episode and a 25% reduction in the number of episodes of ST depression per 24hr.

4.3 RESULTS

Both placebo and spironolactone therapy appear to be clinically well tolerated by all patients in this study. There were no significant changes in baseline haemodynamic parameters such as resting heart rate and blood pressure. Plasma potassium and urea were significantly increased with spironolactone compared to placebo after four weeks of treatment (see *Table 4.2*). Only one patient, who was also taking an ACE inhibitor, had significant hyperkalemia (potassium $> 5.5\text{mmol/L}$) and elevation of plasma creatinine ($>300\mu\text{mol/L}$) which necessitated the dose of spironolactone to be halved (25mg). There was a non-significant increase in plasma magnesium levels. Plasma aldosterone levels measured between 6 and 11am rose from $64 \pm 35\text{ pg/ml}$ to $88 \pm 29\text{ pg/ml}$, and from $203 \pm 122\text{ pg/ml}$ to $273 \pm 160\text{ pg/ml}$ on the placebo and spironolactone study days, respectively.

The effects of spironolactone compared to placebo on the various autonomic parameters measured are displayed in *Tables 4.3-4.5*. Arrhythmic activity was unaffected by spironolactone therapy compared to placebo; there were no significant differences in the number of ventricular extrasystoles or non-sustained ventricular tachycardias (NSVT) between the two treatments.

Circadian pattern of heart rate variability

None of the 24 hr average HRV indices (*Table 4.3*) differed significantly between the two treatments although there was a trend toward higher values in the spironolactone group.

The circadian rhythm of the power spectral components normally seen in healthy subjects, appears to be preserved but blunted in our cohort of angina patients

(*Fig 4.1-4.2*). The daytime values of the LF spectral component were significantly higher than the nighttime values (*Fig 4.2*). However there did not appear to be any significant differences between night and day time values for the HF and LF/HF components. Spironolactone did not appear to have any significant effect on the circadian pattern of the power spectral indices compared to placebo. The early morning rise in HR was preserved and unaffected by both treatments (*Fig 4.1*).

Norepinephrine kinetics

The diurnal profile of plasma norepinephrine levels is displayed in *Fig 4.3*. There were no significant differences between the two treatments. Whole body NA clearance and spillover did not appear to be significantly affected by spironolactone therapy compared to placebo (*Table 4.4*).

Circadian pattern of QT dispersion

QT interval dispersion indices were significantly higher during the daytime compared to the sleeping hours (*Figs 4.4*). Spironolactone did not appear to have any significant effect on QT dispersion compared to placebo (*Table 4.3, Fig 4.4*).

Circadian variation of ischaemic events

Of the 27 patients in the study, 11 patients (41%) while on placebo and 9 patients (33%) during spironolactone therapy had holter-documented ischaemic events. There were no significant differences in the total number of ischaemic events between the two treatments (*Fig 4.5*). In particular, the increased incidence of

ischaemic events during the morning hours did not appear to be affected by spironolactone therapy.

Subgroup analysis

As beta-blocker and ACE inhibitor therapies may influence the autonomic tone, separate subgroup analyses were carried out to see if these drug therapies would influence outcomes. A summary of the results is displayed in *Table 4.5*. Neither subgroups taking beta-blocker nor ACE inhibitor demonstrated any significant differences in any of the measured outcomes compared to those that were not.

Table 4.1: Baseline Demographic Characteristics of randomised patients.*Mean \pm SD (% in brackets)*

Characteristics	n=27
Age (yrs)	64 \pm 9
M / F	23 / 4
Weight (kg)	82 \pm 16
Frusemide	14 (52%)
ACE Inhibitor	16 (59%)
Ca antagonist	13 (48%)
Aspirin	21 (78%)
Nitrates	18 (67%)
Beta-blockers	13 (48%)
History of Diabetes Mellitus	2 (7%)
History of Hypertension	3 (11%)

Table 4.2: Effect of Spironolactone and Placebo on resting haemodynamic and biochemical measures after four weeks of treatment.

Mean \pm SD. P <0.05 if statistically significant.

PARAMETER	PLACEBO	SPIRO	P VALUE
HR	63 \pm 11	61 \pm 10	NS
SBP	121 \pm 23	120 \pm 27	NS
K ⁺	4.0 \pm 0.6	4.5 \pm 0.6	0.01
Urea	6.8 \pm 4.6	7.9 \pm 4.4	0.03
Creatinine	117 \pm 28	128 \pm 33	0.04
Mg ²⁺	0.80 \pm 0.09	0.84 \pm 0.04	NS

Table 4.3 **Mean 24 hr Values:** *Effect of Spironolactone or Placebo on Arrhythmias, Heart Rate Variability and QT Dispersion. Mean \pm SD.*

	Parameter	Placebo	Spiro	p-value
Time-domain measures	RR Int	959 \pm 121	952 \pm 155	NS
	HRV Index	32 \pm 9	35 \pm 10	NS
	SDNN	113 \pm 28	125 \pm 23	NS
	SDANN	96 \pm 21	105 \pm 28	NS
	RMSSD	23 \pm 8	23 \pm 9	NS
Frequency	LF (n.u)	68.1 \pm 12	66.3 \pm 14	NS
(Power Spectral) measures	HF (n.u)	25.2 \pm 10	25.0 \pm 11	NS
	LF/HF	5.16 \pm 3.35	4.69 \pm 2.88	NS
24hr arrhythmia analysis	Ventricular	2005 \pm 2122	1895 \pm 1586	NS
	extrasystoles/24hr	2	2	NS
	NSVT(no.pats)			
ST segment analysis	No. ischaemic episodes/24hr	3.9 \pm 0.5	3.8 \pm 0.5	NS
	Total duration of episodes/24hr (min/24hr)	68 \pm 8	61 \pm 11	NS
	Ischaemic burden (mm.min)	100 \pm 31	91 \pm 28	NS
QT Interval and Dispersion variables	QTd	47.9 \pm 12.5	50.4 \pm 10	NS
	QTcd	54.6 \pm 11.5	53.5 \pm 8.5	NS
	QTmax	485 \pm 20	492 \pm 21	NS
	QTcmax	515 \pm 29	526 \pm 35	NS

Table 4.4: Effect of Spironolactone and Placebo on whole body Norepinephrine (NA) spillover and clearance. *Mean \pm SD.*

		Placebo	Spiro	P-value
AM (0700hrs)	NA clearance (l/min)	3.88 \pm 0.8	4.01 \pm 0.7	NS
	NA spillover (ug/min)	1.50 \pm 0.42	1.47 \pm 0.66	NS
PM (1600hrs)	NA clearance	3.68 \pm 0.54	3.49 \pm 0.65	NS
	NA spillover	1.35 \pm 0.51	1.33 \pm 0.72	NS

Table 4.5 Summary tables of subgroup analyses: *Effect of Spironolactone or Placebo on some of the main outcome measures in subgroups of patients taking concomitant beta-blockers and ACE inhibitors. Mean \pm SD.*

Table 4.5a: Beta blocker subgroup analysis

	Parameter	Placebo	Spiro	p-value
On beta-blockers (n=13)	SDNN	129 \pm 33	140 \pm 29	NS
	LF/HF	4.62 \pm 2.71	4.74 \pm 2.22	NS
	No. ischaemic episodes/24hr	3.2 \pm 0.4	3.3 \pm 0.4	NS
	Ischaemic burden	71 \pm 24	65 \pm 20	NS
	QTd	51 \pm 8	48 \pm 11	NS
	NA clearance (am)	3.95 \pm 0.5	4.01 \pm 0.5	NS
	NA spillover (am)	1.44 \pm 0.5	1.42 \pm 0.38	NS
Without beta-blockers (n=14)	SDNN	101 \pm 21	111 \pm 27	NS
	LF/HF	5.71 \pm 2.49	4.65 \pm 1.90	NS
	No. ischaemic episodes/24hr	4.4 \pm 0.7	4.3 \pm 0.5	NS
	Ischaemic burden	120 \pm 30	115 \pm 26	NS
	QTd	45 \pm 14	51 \pm 7	NS
	NA clearance (am)	3.82 \pm 0.4	4.04 \pm 0.6	NS
	NA spillover (am)	1.56 \pm 0.4	1.51 \pm 0.5	NS

Table 4.5b: ACE inhibitor subgroup analysis

	Parameter	Placebo	Spiro	p-value
On ACE inhibitors (n=16)	SDNN	122 ± 22	120 ± 20	NS
	LF/HF	4.88 ± 2.11	4.90 ± 2.60	NS
	No. ischaemic episodes/24hr	4.0 ± 0.3	4.1 ± 0.3	NS
	Ischaemic burden	89 ± 20	100 ± 16	NS
	QTd	50 ± 11	48.5 ± 10	NS
	NA clearance (am)	3.92 ± 0.5	3.97 ± 0.5	NS
	NA spillover (am)	1.38 ± 0.4	1.52 ± 0.6	NS
Without ACE inhibitors (n=11)	SDNN	107 ± 18	128 ± 24	NS
	LF/HF	5.25 ± 2.25	4.62 ± 2.05	NS
	No. ischaemic episodes/24hr	3.7 ± 0.3	3.7 ± 0.2	NS
	Ischaemic burden	107 ± 20	84 ± 16	NS
	QTd	45 ± 8	52 ± 8	NS
	NA clearance (am)	3.85 ± 0.6	4.06 ± 0.4	NS
	NA spillover (am)	1.58 ± 0.5	1.43 ± 0.5	NS

Figure Legends:

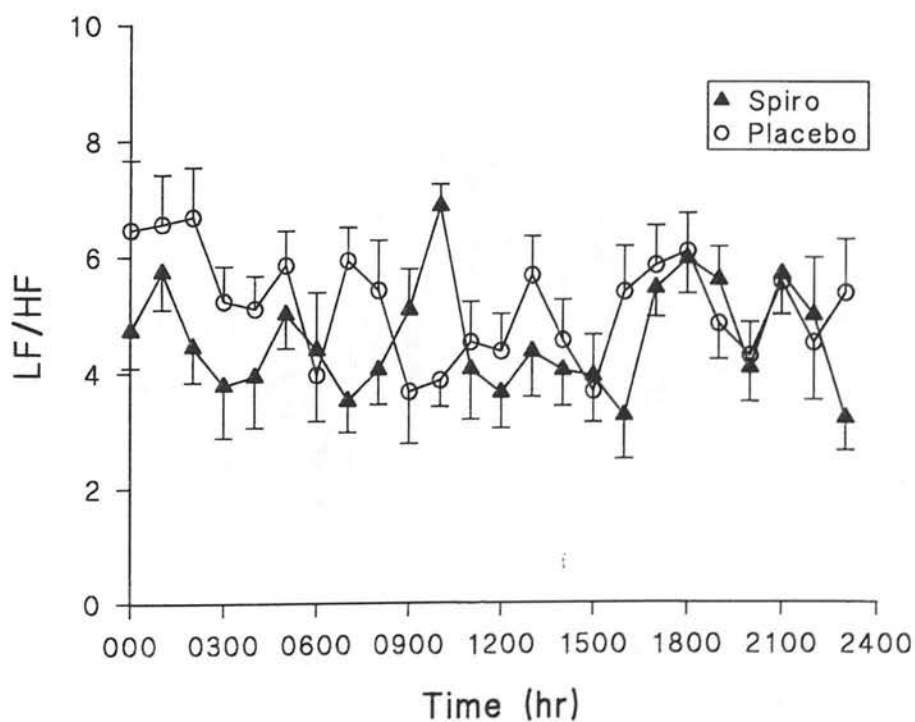
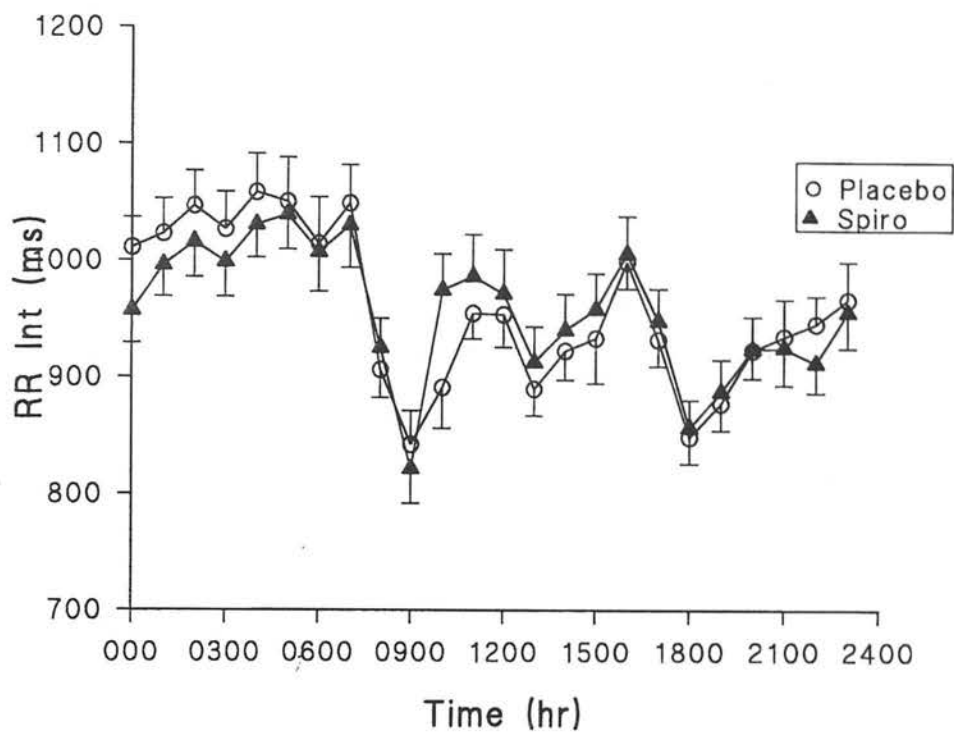
Figure 4.1: Diurnal variation of RR intervals (RR Int) [*top*] and ratio of low frequency to high frequency component (LF/HF) of heart rate variability [*bottom*]. Data expressed as Mean \pm SEM.

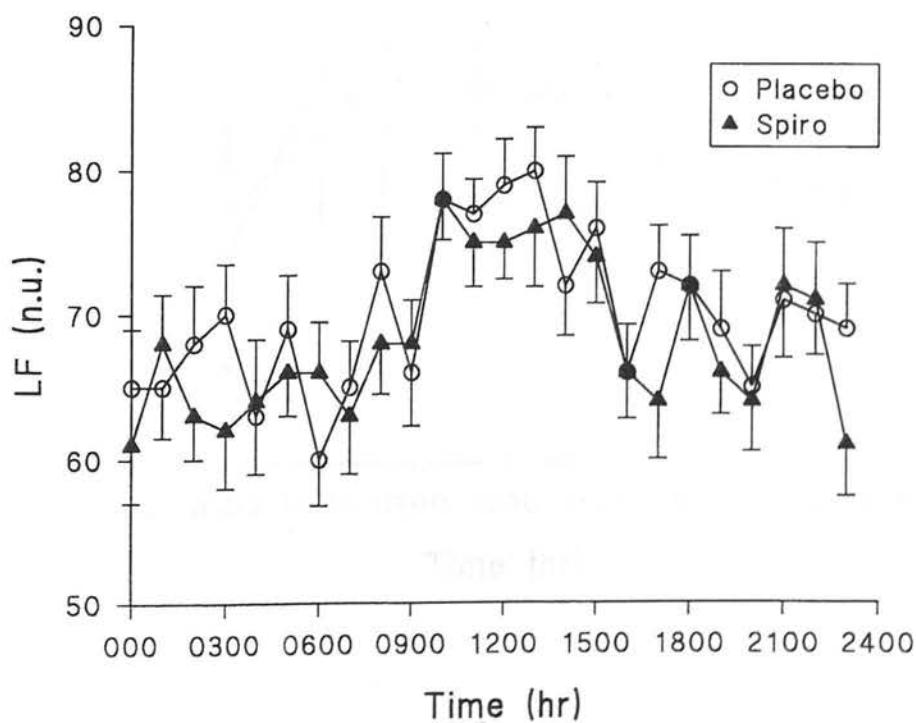
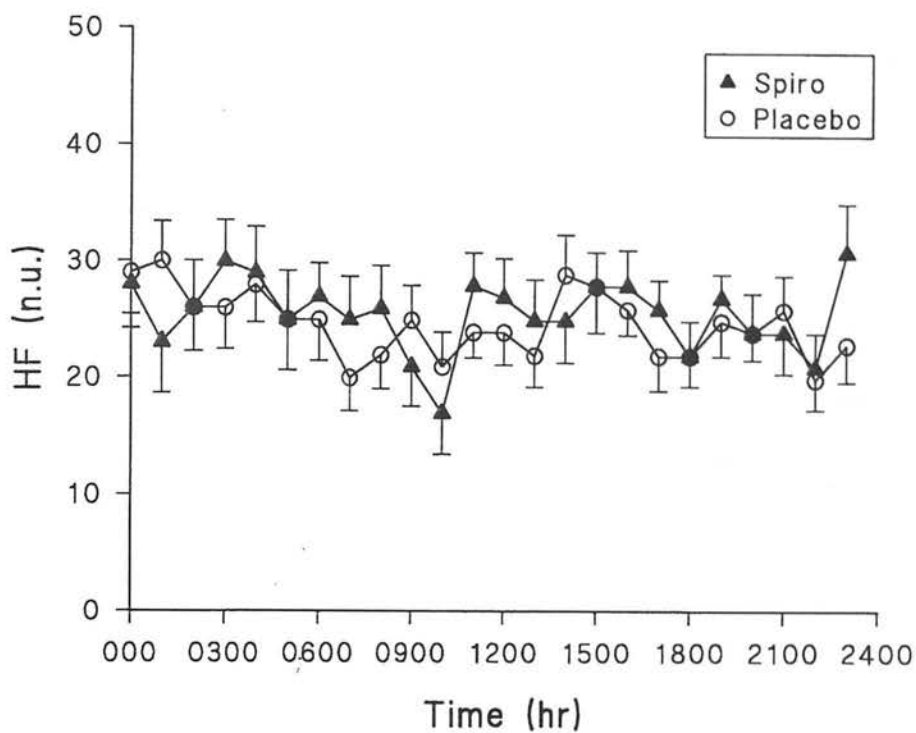
Figure 4.2: Diurnal variation of normalised high frequency component (HF) [*top*] and normalised low frequency component (LF) [*bottom*] of heart rate variability. Data expressed as Mean \pm SEM.

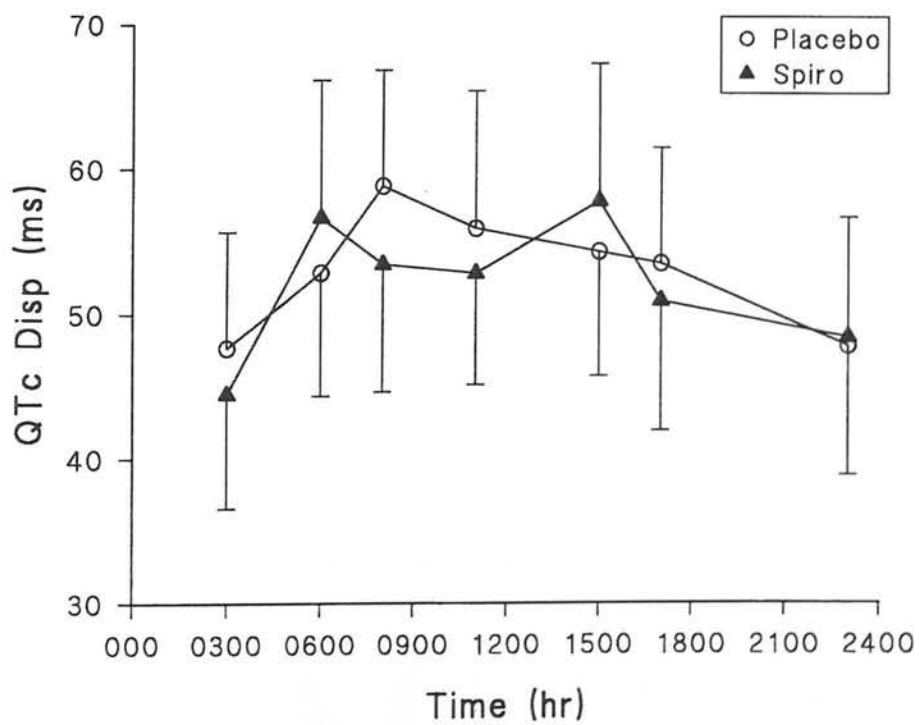
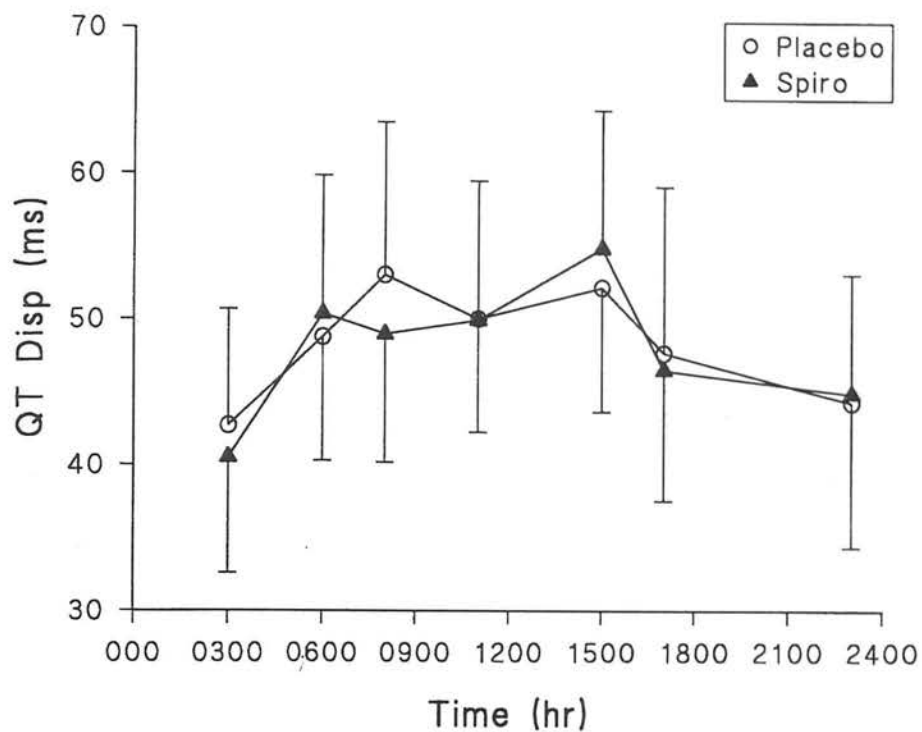
Figure 4.3: Diurnal variation of QT dispersion (QTd) [*top*] and Bazett's rate-corrected QT dispersion (QTc Disp) [*bottom*]. Data expressed as Mean \pm SEM.

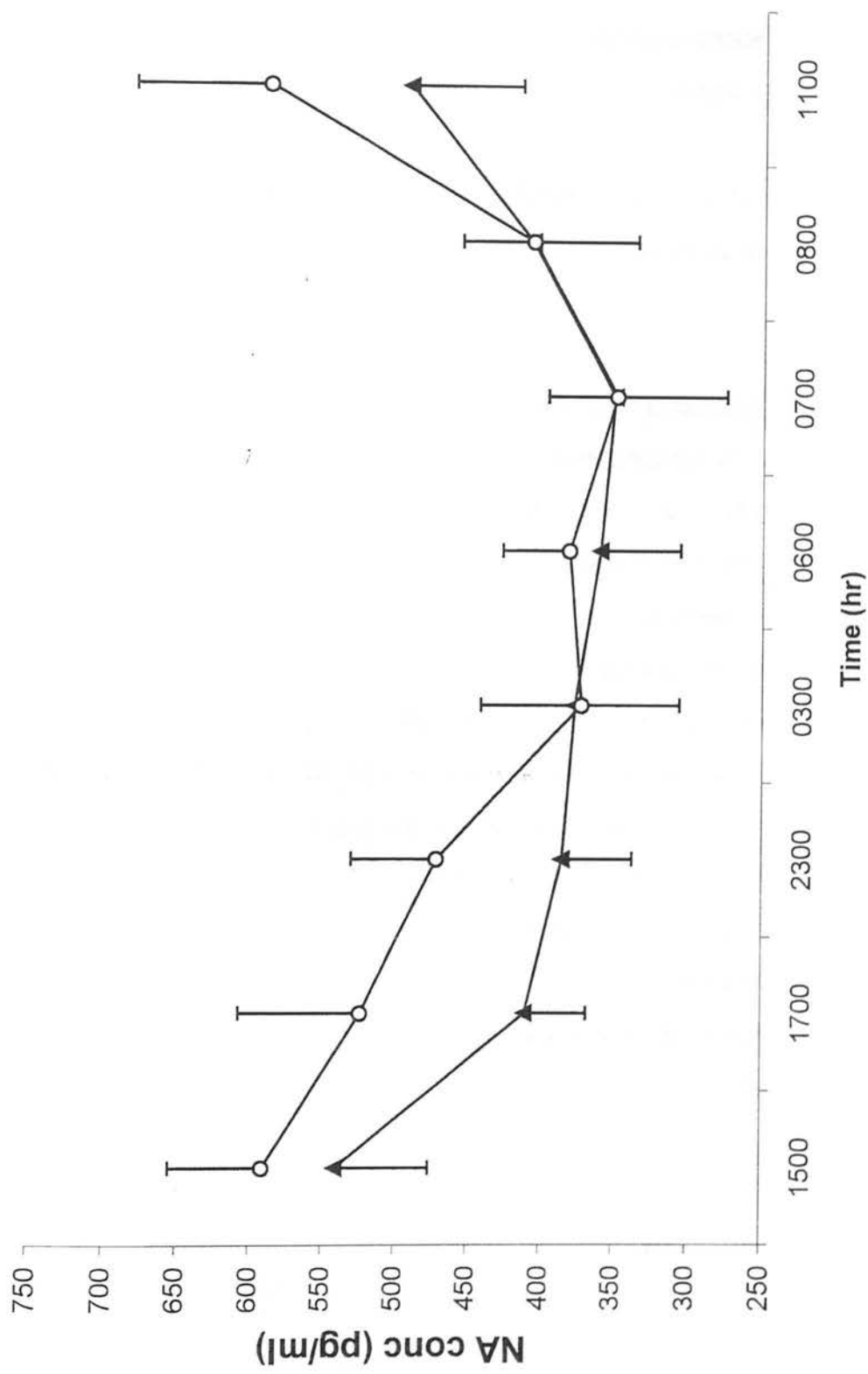
Figure 4.4: Diurnal profile of plasma norepinephrine levels (NA conc). Data expressed as Mean \pm SEM.

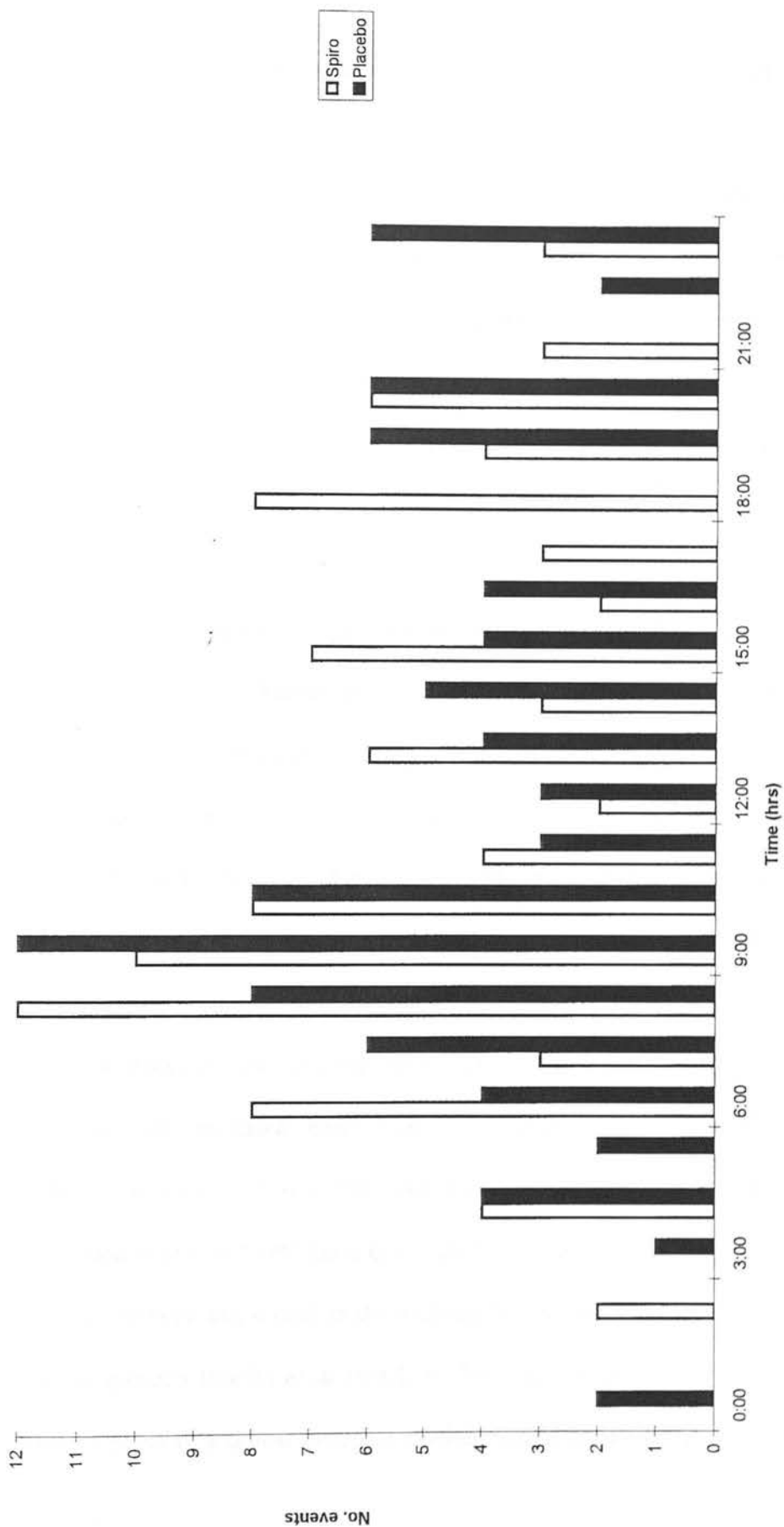
Figure 4.5: Diurnal distribution of ischaemic events.











4.4 DISCUSSION

The present study was designed to assess if the beneficial autonomic modulating properties of spironolactone seen in heart failure also occurred in a different cohort of patients - those with ischaemic heart disease and preserved LV function. In particular, the study was designed to assess if spironolactone-induced bradycardia during the morning hours would translate into a reduction of ischaemic events in patients with chronic stable angina. Spironolactone may have anti-ischaemic effects by way of reducing heart rate at this time. The importance of this observation is that this is the same time of day when ischaemic, arrhythmic and sudden death events are commonest.

The circadian variation of myocardial ischaemia is well established [Muller *et al* 1985, Rocco *et al* 1987, Marchant *et al* 1994]. Studies evaluating 24hr to 48hr Holter ambulatory ECG in patients with stable angina have shown that transient myocardial ischaemic events exhibit a circadian pattern [Rocco *et al* 1987]. Ischaemic episodes peak during the daytime and are associated with a similar circadian rhythm of sympathovagal balance [Marchant *et al* 1994]. The morning peak of cardiovascular events is known to coincide with the surge of sympathetic activity and vagal withdrawal, as reflected by changes in various autonomic and physiological parameters such as increased heart rate and blood pressure, raised plasma catecholamines, increased vascular tone and enhanced platelet aggregability. The sympathetic tone is known to exhibit a circadian rhythm characterised by diminished activity during the night and a peak in the morning hours associated with assumption of the upright position [Furlan *et al* 1990]. ACTH-induced aldosterone secretion is also known to occur in a similar circadian fashion, with its peak secretion occurring

during the morning hours and before upright posture is assumed [Armbruster *et al* 1975].

In this study, the effects of aldosterone blockade on various autonomic and ischaemic markers were assessed in patients with chronic stable angina pectoris. Unlike the RALES trial which focussed primarily on severe CHF patients (NYHA III-IV), the patients recruited here had a normal or near-normal LV function (LVEF >35% and were in NYHA I-II). The addition of a small dose of spironolactone in these patients with chronic stable angina appears to be safe and well tolerated despite the fact that half of them were on concomitant ACE inhibitors. After 4 weeks of treatment, spironolactone caused a small but significant rise in plasma potassium and urea, compared to placebo. These small but significant electrolyte disturbances provide indirect evidence that the subjects were compliant with their study medications.

Spectral analysis of 24hr recordings have shown consistently that in normal subjects, a major change in the autonomic profile occurs at approximately 6am, coinciding with the time of awakening, when a surge of sympathetic activity occurs rapidly and concomitantly with vagal withdrawal [Furlan *et al* 1990]. This is reflected in our diurnal profile of plasma norepinephrine levels (*Fig 4.3*). Consistent with previous studies of patients with stable angina [Marchant *et al* 1994, Huikuri *et al* 1994], a reduced but still preserved circadian pattern in the spectral components is observed.

This is however, the first study to demonstrate that QT dispersion also occurs in a diurnal fashion in patients with ischaemic heart disease, with daytime values being significantly higher than night time values. Although the diurnal pattern of the QTc

interval and dispersion in normal man has been well documented [Molnar *et al* 1997, Ishida *et al* 1997], this has not been previously demonstrated in coronary artery disease.

However, disappointingly in this study, spironolactone therapy did not appear to have any significant influence on the various HRV and QT dispersion measures compared to placebo. Neither norepinehrine spillover nor clearance was affected by aldosterone blockade. The early morning rise in heart rate was still observed in both treatment groups and there were no significant differences in terms of arrhythmic or ischaemic events over the entire 24hr study period.

These findings are in contrast with the previous observations in CHF patients. Why is this so? There are a number of possible reasons. Firstly, the cohort of patients in this study was different from those recruited in the RALES trial. One possibility therefore, is the fact that patients with chronic stable angina and preserved left ventricular function are likely to be less neurohormonally-activated compared to severe CHF patients. This is certainly reflected in our measurements of plasma aldosterone levels during the morning hours. Compared to the cohort of CHF patients described in *Chapter 3*, the morning surge in plasma aldosterone between 6 and 11 am is about a third less in these patients with chronic stable angina. This means that it is possible that we are not seeing the beneficial effects of aldosterone blockade as there is much less circulating aldosterone to antagonize in stable angina patients. Any potential beneficial effect of spironolactone therapy, if at all, may actually be much smaller in magnitude than we had anticipated. For instance, although the incidence of cardiac ischaemic events in our study was in accordance with what we had anticipated [Adamopoulos *et al* 1992, Schuang and Pepine 1977], our study only had a 90%

power at $p < 0.05$ to detect a 25% reduction in the number of episodes of ST depression per day. The study would not have sufficient power to detect reliably an effect of a smaller magnitude (which would in any case, likely to be of doubtful clinical significance).

A second possibility is the fact that unlike the previous studies with CHF patients where the majority of subjects were not on beta-blockers, about half of the patients in our study were. Although beta-blockers are generally accepted as first line anti-anginal therapy, they are not more widely prescribed in our study population (which reflects current practice in the UK) due to concomitant medical conditions (eg chronic obstructive airways disease, peripheral vascular disease etc) or intolerance to their side-effects. This class of drugs has been shown to improve heart rate variability and reduce myocardial ischaemia [Niemela *et al* 1994, Arnim *et al* 1995], and may therefore distort the outcome of the study. However this explanation is unlikely as subgroup analysis did not show any significant differences in outcomes between those taking beta-blockers and those who were not (*Table 5a*).

A third possibility is that the diuretic effect of spironolactone itself may lead to reduced afterload and subsequent activation of neurohormones such as Angiotensin II. Unlike the CHF patients in our previous studies where spironolactone was given *in addition* to ACE inhibitor therapy, only about half the subjects in our study were on an ACE inhibitor. This means that it is possible that a large proportion of the subjects in this study will have activated Ang II that is unopposed, following the administration of spironolactone. The well-known detrimental autonomic effects of unopposed Ang II [Guo and Abboud 1984, Mace *et al* 1985] may theoretically neutralise any beneficial effects obtained from direct aldosterone antagonism. However, subgroup

analysis comparing those already taking ACE inhibitors with those who were not did not reveal any significant differences in outcomes (*Table 5b*).

Study limitations:

The technical limitations described in *Chapter 3* also apply to this study. In particular, the technical difficulty of obtaining a precise and reproducible determination of the QT interval, especially the end of the T wave, is well recognised. Care has been taken to use consistent criteria to define the end of the T wave and to exclude U waves. Although these methodological concerns are well appreciated, they were not a major issue for this study as we were looking at treatment induced *intraindividual* changes in QTd (rather than interindividual changes, which is the usual situation in previous case control studies).

Conclusions

The clinical significance of the neuroendocrine activation of the renin-angiotensin-aldosterone system (RAAS) in the pathophysiology of heart failure is well established. However recent published data from the landmark HOPE trial suggest that inhibition of the RAAS may have far wider clinical implications than we had anticipated. The HOPE findings indicate that *all* patients with a history of cardiovascular disease, not just those with heart failure, may potentially benefit from ACE inhibitors.

In this study, I had examined whether aldosterone blockade too would benefit those cardiac patients with normal LV function. However, the results of the study were disappointing - unlike patients with CHF, aldosterone blockade did not appear

to have any significant beneficial effect in patients with stable angina pectoris. The reasons remain unclear and may be multifactorial. The results of this study highlight, as often the case is with medicine, what we find in one disease state is not found in another. Had the study been positive, it would have provided a strong impetus for a mortality study with spironolactone in angina. As the case turns out, the findings in this study suggest that such a mortality trial would probably not be worth pursuing.

CHAPTER FIVE

ALDOSTERONE AND THE BAROREFLEX

RESPONSE IN MAN

5.1 INTRODUCTION:

The effects of aldosterone on the baroreflex response are explored in this chapter. Baroreflex dysfunction is thought to be a key process leading to ventricular arrhythmias and mortality in patients with chronic heart failure (CHF) and in post-myocardial infarction patients [La Rovere *et al* 1998, Osterziel *et al* 1995]. Indeed, it has even been highlighted that augmentation of the parasympathetic limb of the baroreflex may be an exciting new therapeutic possibility [Townend and Littler 1995]. In animal studies, vagal stimulation has been shown to dramatically improve survival and reduce arrhythmias after coronary artery ligation [Myers *et al* 1976, Zuanetti *et al* 1987].

It is well established that Angiotensin II (Ang II) attenuates baroreflex control of heart rate and increases sympathetic activity [Guo and Abboud 1984, Mace *et al* 1985] and that ACE inhibitors are able to improve baroreflex sensitivity (BRS) [Osterziel *et al* 1990, Marakas *et al* 1995]. Recent animal evidence suggest that aldosterone, like Ang II, may possess detrimental autonomic modulating properties. As discussed in *Chapter 1*, it is now being appreciated that aldosterone too may influence the baroreflex, irrespective of Ang II. Spironolactone improves heart rate variability (as a measure of parasympathetic activity) in CHF patients (*Chapter 3*). Although it remains inconclusive if this was a direct effect of autonomic modulation by aldosterone blockade or due to the diuretic effect of spironolactone (which may potentially reduce right atrial stretch and improve heart rate variability by mechanoelectric feedback [Horner *et al* 1996]), there is experimental data to suggest that aldosterone has major direct effects on the autonomic nervous system and the baroreflex. In an animal model, Wang *et al* [1992] showed that aldosterone infusion

directly reduced baroreceptor discharge from the carotid sinus in dogs.

However, no direct information has yet been reported on the effects of aldosterone on baroreflex responses in man. As with all animal studies, it is essential to determine if these observations also occur in man, especially since baroreceptor dysfunction is known to play a central regulatory role in the development of cardiac arrhythmias [La Rovere *et al* 1998, Osterziel *et al* 1995]. Thus, this study is designed to test the hypothesis that aldosterone attenuates the baroreflex control of heart rate *in vivo* in normal man. I have examined the effects of an acute intravenous aldosterone infusion on the vagal and sympathetic limbs of the autonomic nervous system, by assessment of blood pressure and reflex heart rate responses to phenylephrine (PE), a vasopressor agent, and to sodium nitroprusside (SN), a vasodilator.

5.2 METHODS

Subjects and Protocol

14 normal male volunteers [mean age (SD) 25 (9)] were studied on 2 separate days, at least 72 hours apart, in a placebo-controlled, randomized, double-blind, cross-over fashion. Subjects were asked to refrain from alcohol, caffeine and cigarettes for 24 hrs and to fast for 2 hrs before each study day.

Subjects rested quietly in the supine position throughout the study. Two 18G intravenous cannulae were inserted into forearm veins, one in the right arm for blood sampling and one in the left arm for infusion of either aldosterone or 5% dextrose as vehicle. After 45 minutes of bedrest, baseline values of blood pressure (BP) and heart

rate (HR) were measured non-invasively in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, USA) with the cuff being placed around the subjects left arm. 12 lead electrocardiograms and venous blood (15mls) for baseline aldosterone and angiotensin II assays were also obtained.

Following this, a continuous infusion of either vehicle (5% Dextrose) or d-Aldosterone (Tayside Pharmaceuticals) in similar volumes was commenced in the left arm. The d-aldosterone was infused at a rate of 12pmol/kg/min. After 45 minutes into the infusion, further triplicate recordings of blood pressure, heart rate and continuous 12-lead ECGs were obtained along with blood samples for aldosterone and angiotensin II assays.

The haemodynamic study was then started ie. after 90 mins of supine bed rest and after 45 mins after commencement of the infusion of aldosterone or vehicle. The reflex baroreflex response to a vasopressor agent, phenylephrine (PE), was assessed in the first half of the study. Intravenous PE was given by infusion into the right forearm. It was administered in stepwise 10 min infusions (0.2-3.6 ug/kg/min) by use of an infusion pump (IMED, San Diego, CA). The infusion was stopped when a 35-40 mmHg rise in systolic arterial pressure had been achieved. The average systolic BP, HR and R-R interval obtained from continuous ECG recordings between 8 and 10 mins after each infusion dose were recorded.

After completion of these measurements with PE, HR and BP were allowed to return to baseline values for 30 minutes before the second phase of the study was commenced with intravenous sodium nitroprusside given in stepwise 5 minute infusions (0.2-5.2ug/kg/min) until a maximum drop of systolic BP of 25mmHg was

achieved. HR, BP and ECG recordings were obtained after 4-5 minutes of each infusion dose.

Baroreflex Sensitivity (BRS) Assessment

The R-R intervals were plotted against the systolic blood pressure in a graph, and a computerised curve fit was then carried out to establish a linear portion of the line of best fit. Separate linear regression lines were plotted for the responses to phenylephrine and sodium nitroprusside. Traditionally, the slope derived from the linear regression line ($\Delta RR/\Delta SBP$) obtained from the vasopressor (PE) half of the haemodynamic study has been used as an index of baroreflex sensitivity (BRS). As in previous studies [4,11], only regression lines that had a correlation coefficient of >0.8 were used. Full description of the methodology is described in *Chapter 2*.

Aldosterone And Angiotensin II Assays

As described in *Chapter 2*.

Statistical Analysis

All data were analysed using the Statgraphics software package (STSC Software Publishing Group, Rockville, MD, USA) as described in *Chapter 2*. Analysis of variance at each dose increment, using subjects and treatment as within factors, and Bonferroni multiple range tests were performed to determine the significance of the effects of aldosterone on the haemodynamic response to phenylephrine and sodium nitroprusside. The relationships between R-R intervals and systolic BP were studied by correlation and linear regression analyses; BRS between treatment groups were

analysed using the paired Student's *t* test.

5.3 RESULTS

Baseline Measurements (See Table 5.1)

No significant differences in baseline measurements were seen between vehicle and aldosterone infusion days. A significant rise in plasma aldosterone levels was noted after 45 mins of aldosterone infusion compared to placebo. There was a non-significant decrease in Ang II levels following aldosterone infusion compared to vehicle.

Haemodynamic Measurements (See Fig.5.1-5.5, Table 5.2)

No significant changes in resting blood pressure and heart rate recordings were observed during aldosterone infusion compared to vehicle. Systolic blood pressure increased and decreased in a stepwise fashion in response to the phenylephrine and sodium nitroprusside infusions respectively in both groups; no significant differences in BP responses were observed in the aldosterone group compared to vehicle. Similarly, no significant differences in reflex heart rate responses to sodium nitroprusside were observed between the two groups. The slopes of the linear regression line in response to sodium nitroprusside ($\Delta RR/\Delta SBP$) were not significantly affected by aldosterone compared to vehicle [8.62 ± 4.64 ms/mmHg (Mean \pm SD) vs 9.07 ± 2.11 ms/mmHg; $p=0.7$] (See Fig 5.5). However, reflex heart rate responses to phenylephrine were significantly impaired in the aldosterone group compared to vehicle ($p<0.05$). BRS was significantly depressed in the aldosterone group [8.36 ± 2.19 ms/mmHg vs 10.12 ± 2.27 ms/mmHg ; $p<0.04$].

TABLE 5.1: Baseline values. Results are expressed as means \pm SD. Statistical significance : *P<0.05 compared with vehicle.

	Vehicle	Aldosterone
Heart rate (beats/min)	61 (8)	60 (9)
Systolic blood pressure (mmHg)	114 (10)	116 (5)
R-R Interval (msec)	999 (156)	1021 (181)
Aldosterone- baseline levels (pg/ml)	115 (107.8)	106.1 (81.8)
Aldosterone level- 45 mins after start of infusion (pg/ml)	72.6 (62.6)	489.8 (83.3)*
Angiotensin II levels – 45 mins after start of infusion (pg/ml)	15.2 (7.6)	10.4 (3.5)

TABLE 5.2: Change in haemodynamic parameters in response to phenylephrine infusion. Values are mean and 95% confidence intervals

(in brackets). Statistical significance : *P<0.05, †P<0.01 compared with vehicle.

Vehicle				Aldosterone			
Dose	PE	ΔsBP	ΔHR	ΔR-R Int	ΔsBP	ΔHR	ΔR-R Int
(ug/kg/min)		(mmHg)	(b/min)	(msec)	(mmHg)	(b/min)	(msec)
0.2		3.5 (0.6 to 6.4)	3.2 (1.9 to 4.5)	57.9 (31.9 to 83.8)	3.6 (0.7 to 6.6)	2.7 (1.4 to 4.0)	50.2 (24.2 to 76.2)
0.6		12.5 (8.1 to 16.9)	8.3 (6.1 to 10.6)	163 (120 to 206)	13.5 (9.0 to 17.9)	3.5 (1.3 to 5.8)†	63.9 (20.7 to 107)†
1.2		20.5 (14.4 to 26.6)	13.9 (12.1 to 15.8)	288 (247 to 330)	24.6 (18.7 to 30.6)	9.0 (7.1 to 10.8)†	182 (141 to 222)†
2.4		43.1 (36.3 to 49.8)	19.0 (17.5 to 20.5)	435 (385 to 485)	44.1 (37.4 to 50.9)	15.3 (13.8 to 16.8)†	343 (293 to 393)*
3.6		57.2 (51.7 to 62.7)	22.1 (20.1 to 24.2)	571(533 to 609)	60.4 (53.9 to67.0)	18.8 (16.4 to 21.2)*	462 (416 to 507)†

Figure Legends:

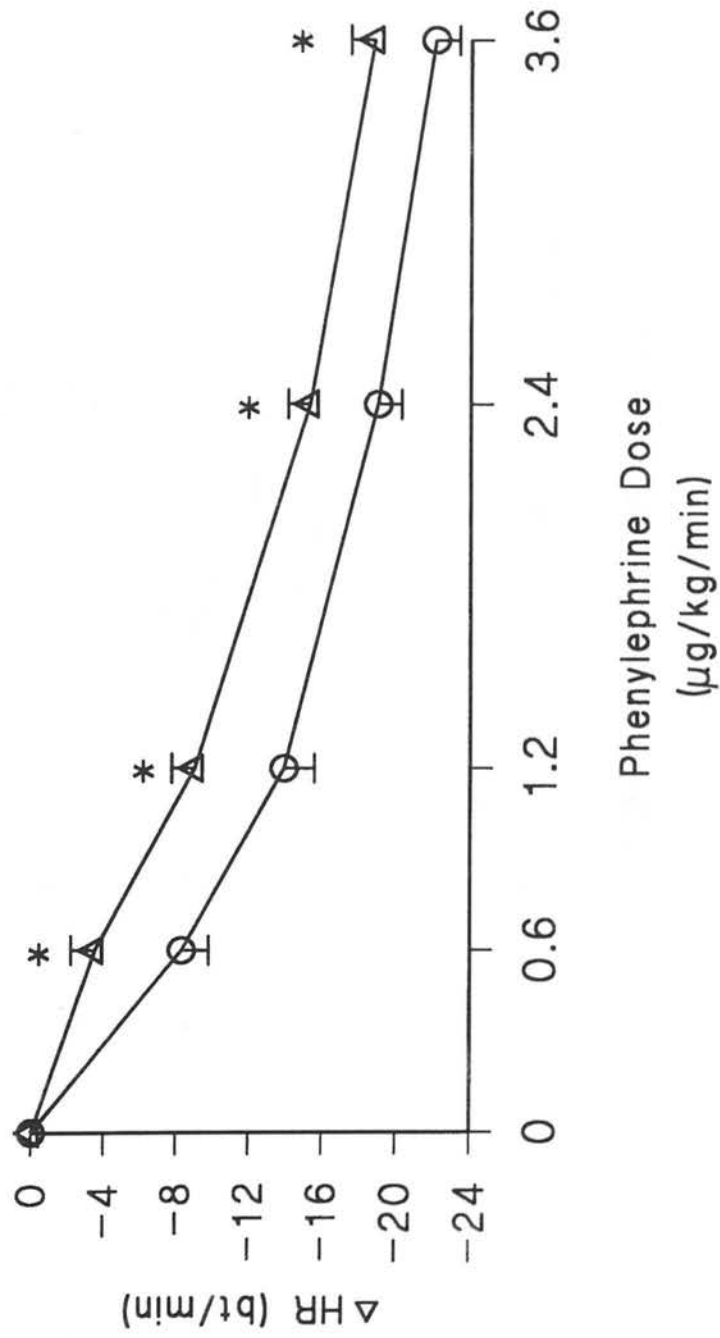
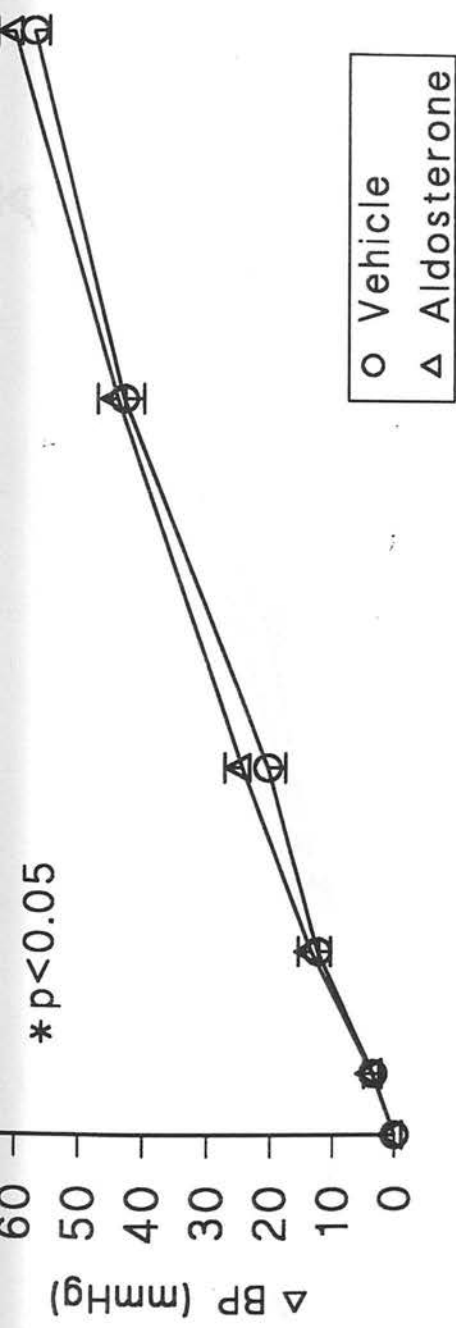
Fig 5.1: Change in Heart Rate (Δ HR) and Blood Pressure (Δ BP) responses to incremental infusions of Phenylephrine. Values are mean \pm SEM. * $p<0.05$ compared with placebo.

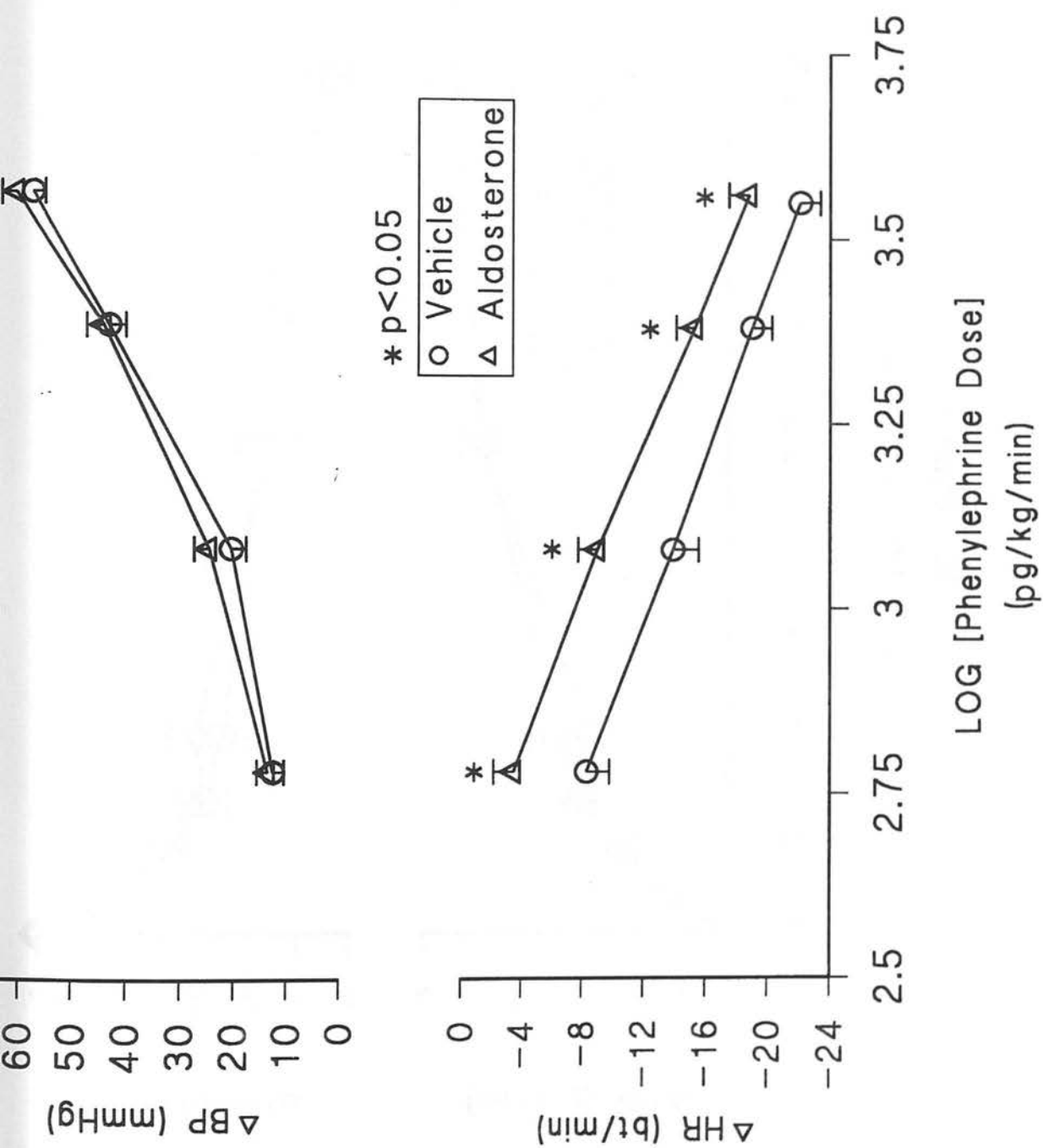
Fig 5.2: Log Phenylephrine Dose-Response curve. Change in Heart Rate (Δ HR) and Blood Pressure (Δ BP) responses to incremental infusions of Phenylephrine. Values are mean \pm SEM. * $p<0.05$ compared with vehicle.

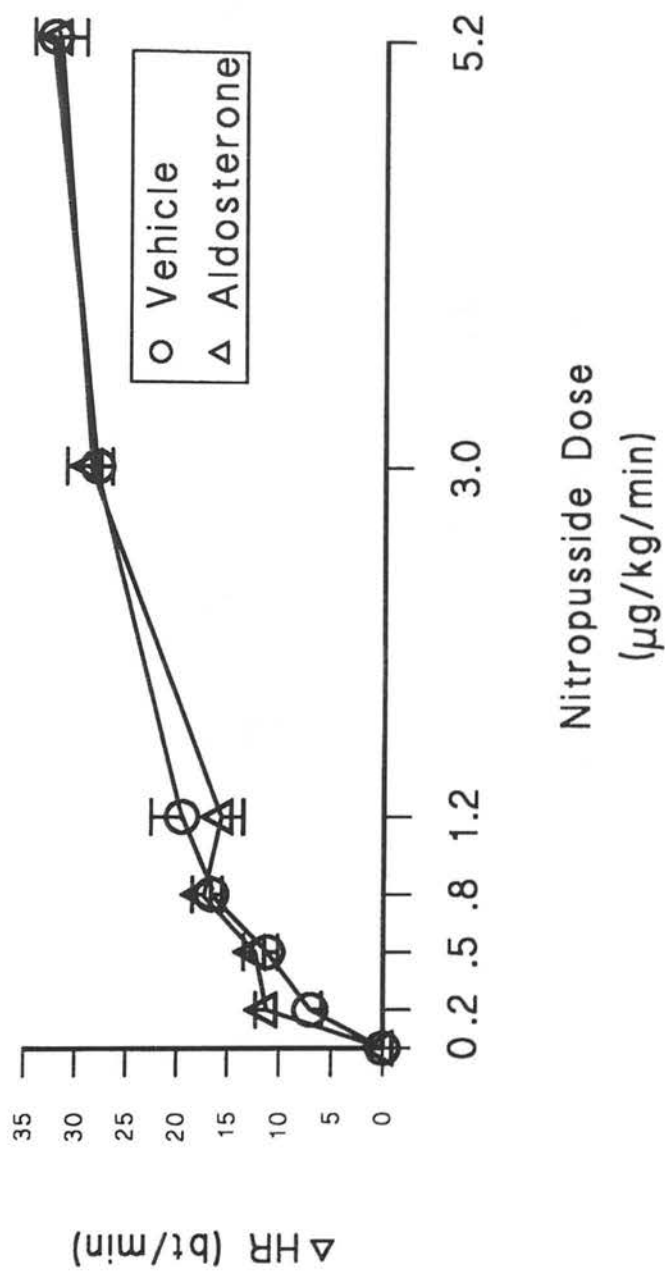
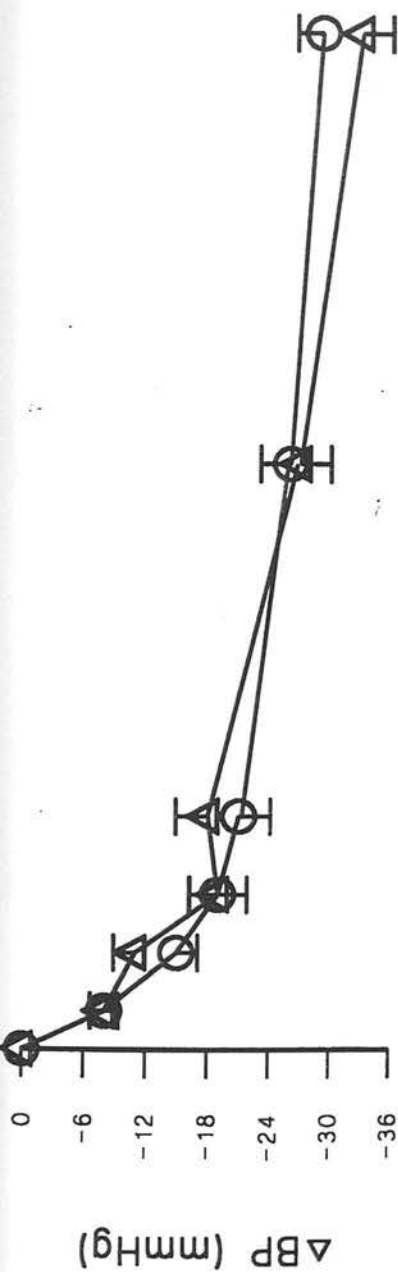
Fig 5.3: Change in Heart Rate (Δ HR) and Blood Pressure (Δ BP) responses to incremental infusions of Sodium Nitroprusside. Values are mean \pm SEM.

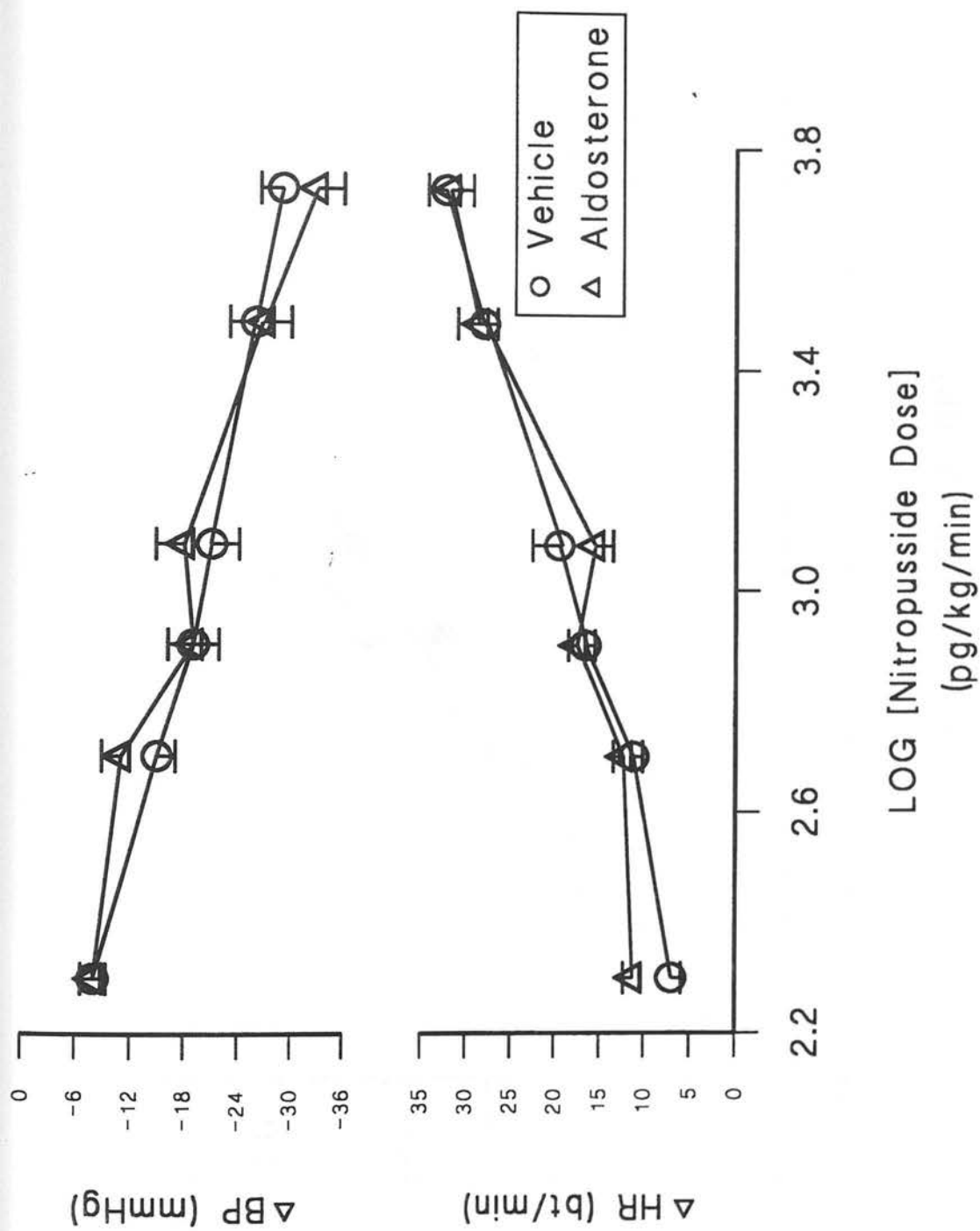
Fig 5.4: Log Sodium Nitroprusside Dose-Response curve. Change in Heart Rate (Δ HR) and Blood Pressure (Δ BP) responses to incremental infusions of nitroprusside. Values are mean \pm SEM.

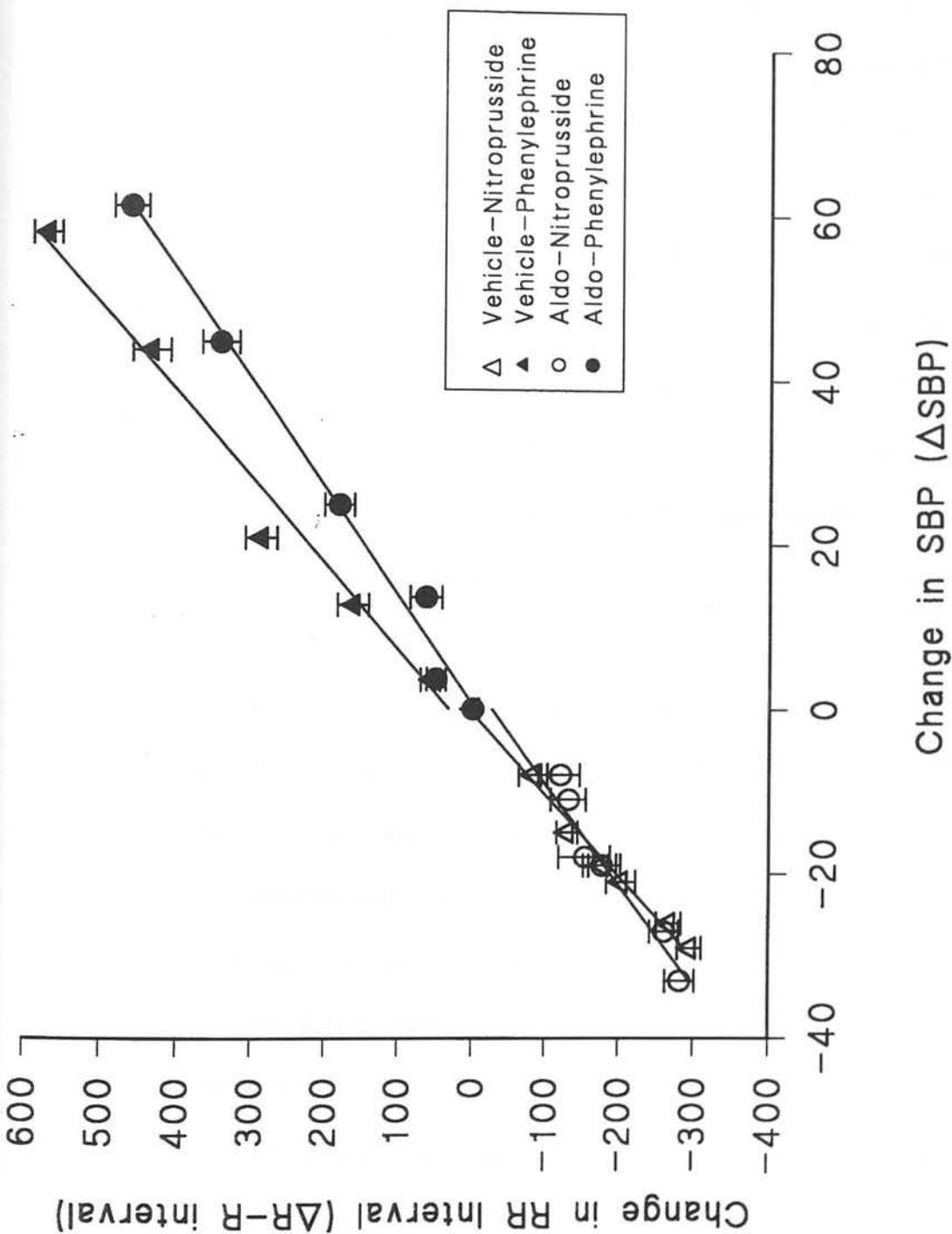
Fig 5.5: Relationship between changes in RR Interval (Δ R-R Int) and systolic Blood Pressure (Δ SBP). Values are mean \pm SEM. Separate linear regression lines for responses to phenylephrine and sodium nitroprusside for the two treatments are shown.











5.4 DISCUSSION

This study confirms previous observations from animal models that aldosterone impairs the baroreflex response. The sensitivity of the arterial baroreceptors to a change in BP is an important determinant of the ability of the autonomic nervous system to maintain cardiovascular homeostasis. Increases in arterial BP in response to pressor stimuli such as PE, activate mechanosensitive nerve endings in the carotid sinus and aortic arch, which then send sensory information to the central nervous system via the glossopharyngeal and vagus nerves. This in turn, results in increased reflex vagal efferent outflow to the heart. The administration of a hypotensive agent such as Sodium Nitroprusside (SN), on the other hand results in vasodilatation and generalised unloading of arterial and cardiopulmonary baroreceptors leading to increased cardiac sympathetic activity [Newton and Parker 1996, Shepherd *et al* 1981].

In this study, aldosterone has been shown to impair heart rate response to PE. This is in fact, in keeping with a previous observation that aldosterone halved the bradycardic response to infused noradrenaline, another vasopressor agent [Barr and Struthers 1994]. Furthermore, it is also interesting to note that aldosterone does not appear to have any significant effect on the reflex tachycardic (or cardiac sympathetic) response to SN. These data together suggest that aldosterone exerts major effects on the parasympathetic limb of the autonomic nervous system.

With regards to its specific mechanism of action, there is evidence from animal studies to suggest that aldosterone may have a direct action on the arterial baroreceptors. Wang *et al* [1992] showed not only that aldosterone reduces the HR response to changes in BP, but they also showed conclusively that aldosterone

directly reduces baroreceptor discharge from the carotid sinus of dogs. These effects were seen with both acute and chronic administration of aldosterone [Wang 1994]. On a cellular level, the mechanism is less certain. Aldosterone may elicit part of its effect on the arterial baroreceptors by stimulation of the Na-K-ATPase activity [Wang 1994] as aldosterone is known to be a potent Na-K-ATPase stimulant [Blot-Chabaud *et al* 1990, Verrey *et al* 1989]. Depressed baroreceptor function following chronic aldosterone infusion in animal models has been shown to be partially reversed with a bolus injection of the Na-K-ATPase inhibitor, ouabain [Wang 1994]. Similarly, digoxin, another cardiac glycoside / Na-K-ATPase inhibitor has also been shown to improve BRS in chronic hyperaldosteronemic states such as CHF [Ferrarri *et al* 1981]. However, such an effect of ouabain was not seen following acute administration of aldosterone [Wang *et al* 1992] suggesting that other mechanisms may be responsible for the acute effects of the hormone. Interestingly, the impaired baroreceptor function due to acute administration of aldosterone could be prevented by denudation of the endothelial cells in the carotid sinus area, which has led to the suggestion that aldosterone may either stimulate endothelial cells to release an unknown substance that depresses BRS activity or inhibit endothelial cells from releasing a substance such as nitric oxide which may enhance BRS activity; the latter would be consistent with the recent observations of Farquharson and Struthers [2000] where aldosterone blockade was found to increase nitric oxide bioactivity and improve endothelial vasodilator dysfunction in CHF patients.

Although this study was not designed to determine the specific mechanism by which aldosterone impairs the baroreflex, it has nonetheless, important clinical implications as it is the first study as such to extend these observations to man. This

study adds to the growing body of evidence that aldosterone, like Ang II, has autonomic modulating properties as discussed in previous chapters. Aldosterone has been shown to block norepinephrine uptake in the heart *in vivo* in an animal model [Barr *et al* 1995]. In accordance with this, spironolactone, an aldosterone antagonist, increased myocardial norepinephrine uptake [Barr *et al* 1995] and improved heart rate variability in patients with CHF (see *Chapters 3 and 4*). Although in this study, aldosterone did not appear to have any significant effect on the reflex sympathetic response to SN in healthy volunteers, this finding is not necessarily a contradiction to previous findings observed in heart failure patients since infusing nitroprusside into normal man clearly does not exactly reproduce the activated sympathetic system of CHF patients, even though nitroprusside is a traditional way of assessing sympathoactivation in man. One important difference between the two is that filling intracardiac pressures are high in CHF but are likely to be subnormal after nitroprusside unloading in normal man.

Study limitations

There are however, some limitations to the study worth discussing. Firstly, the study was performed in healthy subjects and not in patient groups. CHF patients for instance, have a markedly abnormal and complex haemodynamic and neurohormonal state. Although it would be of interest to assess the effects in these patient groups, they are not ideal for the purpose of this study which was to assess the effects of aldosterone on the baroreflex response *in vivo* in man. In this study of healthy subjects we were able to purely, isolate the effects of aldosterone on the baroreflex (and hence, confirming the data from animal models) while avoiding the many possible

confounding factors found in CHF patients who are characterised by the presence of other circulating neurohormones, impaired vascular tone and endothelial dysfunction.

Secondly, infusions rather than boluses of pressor and vasodepressor stimuli were used in this study, allowing for baroreceptor “resetting” to occur and hence reducing or dampening any change in baroreceptor response. This may mean that small changes in baroreflex response to SN, for instance, may have gone undetected. On the other hand, one might expect, if anything, the blunting effect of aldosterone on the baroreflex response to PE to be amplified if the bolus method had been used instead. BRS measurements by infusion method may not be equivalent to the bolus method, but the infusion method has been shown to be reproducible [Sullebarger *et al* 1990].

As mentioned in *Chapter 2 (Methods)*, infusions were used in this study firstly, as it allowed us to monitor HR and BP changes at steady state non-invasively at each incremental dose which has the advantage that our readings were taken in triplicate, which should minimise random measurement error. Secondly, the bolus method requires either invasive intra-arterial measurements (more risk and discomfort to the subject using radial artery devices) or non-invasive beat-to-beat analysis to be performed using the FINAPRES but this is not possible at our institute as the FINAPRES devices are no longer available in the United Kingdom. Furthermore we were more interested in *changes or differences* in baroreceptor sensitivities between two treatments rather than in using absolute levels of BRS to compare one population with another.

Conclusion

In summary, this study has established that in man *in vivo* aldosterone has a detrimental effect on the parasympathetic component of the baroreflex response. The effects of aldosterone on the autonomic nervous system have important clinical implications. It is noteworthy that in our study, the plasma concentration of aldosterone [mean (SD) 489.8 (83.3) pg/ml] achieved on the treatment days were similar to those observed in CHF patients [Struthers 1996]. A reduced BRS is a marker of high risk patients in both CHF [Osterziel *et al* 1995] and IHD [La Rovere *et al* 1998]. Therefore, as baroreflex dysfunction predisposes the myocardium to arrhythmias, the findings of this study may explain some of the beneficial effects seen with spironolactone in the clinical studies described in the previous two chapters and also for the mortality reduction (particularly sudden deaths) seen in the multicentre RALES study. [Pitt *et al* 1999].

CHAPTER SIX

ENDOGENOUS ANGIOTENSIN II AND BARORECEPTOR DYSFUNCTION:

A COMPARATIVE STUDY OF LOSARTAN AND ENALAPRIL IN MAN

6.1 INTRODUCTION

It is well established that Angiotensin II (Ang II) attenuates baroreflex control of heart rate and sympathetic activity [Guo and Abboud 1984, Mace *et al* 1985]. Indeed, the effect of ACE inhibitors on BRS may well be instrumental in their ability to improve mortality in heart failure. Compared to ACE inhibitors, Ang II type 1 receptor (AT1) antagonists offer a more selective and complete blockade of Ang II. The question naturally arises whether AT1 receptor antagonists will also have favourable effects on BRS.

Pharmacologically, they are an attractive class of drugs as they work at the receptor level, and should therefore avoid the neurohormonal “escape” phenomenon seen with ACE inhibitors resulting from Ang II production by non-ACE dependent pathways [Urata *et al* 1990]. Therefore they theoretically, may even be more effective at boosting BRS because Ang II levels are not completely suppressed by ACE inhibitors. However, although direct AT1 receptor antagonists have been shown to improve baroreceptor function in animal models [Moreira *et al* 1994, Oliveira *et al* 1996], the only study to examine this question in man found no effect of losartan on baroreflex sensitivity [Rongen *et al* 1998]. This whole question has been given added impetus by the Evaluation of Losartan in the Elderly (ELITE I) trial results. Although designed primarily to assess safety and efficacy of the treatments and not mortality, it was intriguingly observed in the multicentre ELITE I trial [Pitt *et al* 1997] that those randomised to losartan had a 46% reduction in all-cause mortality in comparison to captopril-treated patients, which was primarily due to a decrease in sudden cardiac deaths*.

The purpose of the present study, which was designed following the

publication of the intriguing ELITE I results, was two folds. Firstly, the main aim was to assess if AT1 receptor antagonism really does improve baroreceptor function in man, as had been previously demonstrated in animal models although it did not appear to in the only human study of the matter [Rongen *et al* 1998]. The secondary aim was to perform a head to head comparison of direct Ang II blockade at the receptor level with that of non-specific ACE inhibition to see if there were any major differences between them which might be relevant to the ELITE* results. In this study, a comparison of a single oral dose of losartan potassium 50mg was made with a single dose of an ACE inhibitor, enalapril maleate 20mg. In particular, this study was performed in normal man in whom the endogenous renin angiotensin system (RAAS) had been activated as it would enable us to assess the activated RAAS of heart failure in isolation while avoiding the confounding influences of increased age, co-morbidity and polypharmacy which would be present in CHF.

** [NB -The present study was designed, conducted and published prior to the publication of the ELITE II trial results. In the latter study, which was a much larger trial compared to ELITE I and had mortality as a primary end-point, losartan did not appear to concur any survival benefit over that of captopril. The implications of the findings of the present study in the light of ELITE II, are discussed further in the Discussion section.]*

6.2 METHODS

Subjects And Protocol

10 normal male volunteers [mean age (SD) 23 (6.9)] were studied on 3 separate days, 10 days apart, in a placebo-controlled, randomized, double-blind,

cross-over fashion. Three days prior to each study visit, subjects were pre-treated with oral frusemide 40mg/ day to activate their endogenous renin-angiotensin system. The subjects were instructed to take the daily Frusemide dose at 1800hr and they were asked to maintain their usual diet for the duration of the study and to adhere to the same pattern of meals in the 48hr preceding each visit day. Subjects were also required to refrain from alcohol, caffeine and cigarettes for 24 hrs and to fast for 2hrs before each study day.

On the study day (ie. the day after they had completed each course of frusemide tablets), the subjects attended our department at 8am. Each subject was given randomly, a different tablet on each visit day, which comprised of either a placebo tablet, enalapril 20mg or losartan 50 mg.

Subjects rested quietly in the supine position throughout the study period. An 18G intravenous cannula was inserted into a right forearm vein for drug infusions and blood sampling. After 45 minutes of bedrest, baseline values of blood pressure (BP) and heart rate (HR) were measured non-invasively in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, USA) with the cuff being placed around the subjects left arm. 12 lead electrocardiograms and venous blood (15mls) for baseline aldosterone and Ang II assays were also obtained.

The haemodynamic and baroreceptor assessments were carried out after 6 hrs following ingestion of the oral medication as the haemodynamic effects of both a single dose of oral losartan and enalapril are known to peak after 6 hrs [Brunner *et al* 1981, Ohtawa *et al* 1993]. Further triplicate recordings of resting blood pressure, heart rate and continuous 12-lead ECGs were obtained before the reflex baroreceptor

response to a vasopressor agent, phenylephrine (PE), was assessed. Intravenous PE was administered in stepwise 10 min infusions (0.2-3.6 ug/kg/min) by use of an infusion pump according to the protocol as detailed in *Chapter 2: Methods*. The infusion was stopped when a 35-40 mmHg rise in systolic arterial pressure had been achieved. The average systolic BP, HR and R-R interval obtained from continuous ECG recordings between 8 and 10 mins after each infusion dose were recorded. After completion of these measurements with PE, HR and BP were allowed to return to baseline values.

Baroreflex Sensitivity (BRS) Assessment

The R-R intervals were plotted against the systolic blood pressure in a graph, and a computerised curve fit was then carried out to establish a linear portion of the line of best fit. Full description of the methodology is described in *Chapter 2*. The slope of the linear portion of this relationship ($\Delta RR / \Delta sBP$) was taken as an index of baroreflex sensitivity (BRS).

Aldosterone And Angiotensin II Assays

As described in *Chapter 2*.

Statistical Analysis

All data were analysed using the Statgraphics software package (STSC Software Publishing Group, Rockville, MD, USA). Multiple analysis of variance, using subjects and treatment as within factors, and Bonferroni multiple range tests were performed to determine the significance of the effects of losartan and enalapril

on the haemodynamic response to phenylephrine. The relationships between R-R intervals and systolic BP were studied by correlation and linear regression analyses; BRS between the placebo and treatment groups were analysed using the paired Student *t* test.

6.3 Results

Baseline Measurements (See Table 1)

Resting haemodynamic and biochemical measurements were similar at all study visits prior to administration of the study medications (Table 1). As expected, basal plasma Ang II and aldosterone levels were elevated as a result of frusemide-induced salt depletion. At 6hrs following ingestion of study medication, resting blood pressure was significantly reduced with both losartan and enalapril by 8.4 mmHg (95% CI 4.2, 12.6; $p=0.0038$) and 9.6 mmHg (95% CI 4.6, 14.6; $p=0.004$) respectively, compared to placebo. However there were no significant differences with resting heart rate either at the start or at 6 hrs after medication.

Baroreflex Sensitivity Assessment (See Fig 6.1-6.3, Table 6.2)

Systolic blood pressure and reflex heart rate increased and decreased respectively, in a stepwise fashion in response to the phenylephrine infusion on all three study days. Whereas no significant differences in BP responses were observed with any of the study medications, reflex heart rate responses to phenylephrine were significantly increased with both enalapril and losartan compared to placebo ($p<0.05$). The $\Delta RR / \Delta sBP$ ratio, taken as a measure of BRS was significantly increased with

enalapril [12.2 ± 4.6 ms/mmHg (Mean \pm SD)] and losartan [11.9 ± 3.6 ms/mmHg] compared to placebo [9.2 ± 4.5 ms/mmHg]; ie. enalapril and losartan increased the $\Delta\text{RR} / \Delta\text{sBP}$ ratio by 3.0 ms/mmHg (95%CI 0.5, 5.6; $p < 0.05$) and 2.8 ms/mmHg (95%CI 0.6, 5.0; $p < 0.038$) respectively. There were however no significant differences between losartan and enalapril [mean difference 0.25 (95%CI -1.6, 2.1)]. The individual BRS indices are displayed in Fig 6.3.

Table 6.1: Baseline values. Results are expressed as Means \pm SD. Statistical significance: *p<0.004 compared to placebo.

		Placebo	Enalapril	Losartan
Heart rate (bt/min)	pre-dose	67.6 (4.6)	66.6 (9.1)	69.1 (9.4)
	6 hr post-dose	68.0 (7.4)	67.1 (8.4)	68.8 (7.1)
Systolic BP (mmHg)	pre-dose	117.6 (9.9)	117.1 (7.6)	118.5 (7.1)
	6 hr post-dose	119.3 (8.5)	107.5 (9.8)*	110.1 (7.4)*
R-R Int (msec)	pre-dose	892 (62)	916.5 (122)	883 (115)
	6 hr post-dose	892 (96)	907 (108)	881 (89)
Plasma Ang II (pg/ml)		42.6 (34.4)	40.7 (19.9)	44.9 (29.0)
Plasma Aldo (pg/ml)		194 (189)	113 (63)	141 (79)

Table 6.2: Changes in haemodynamic parameters in response to phenylephrine infusion. Values are Mean \pm SD. Statistical significance: * $p < 0.01$; † $p < 0.05$ compared to placebo.

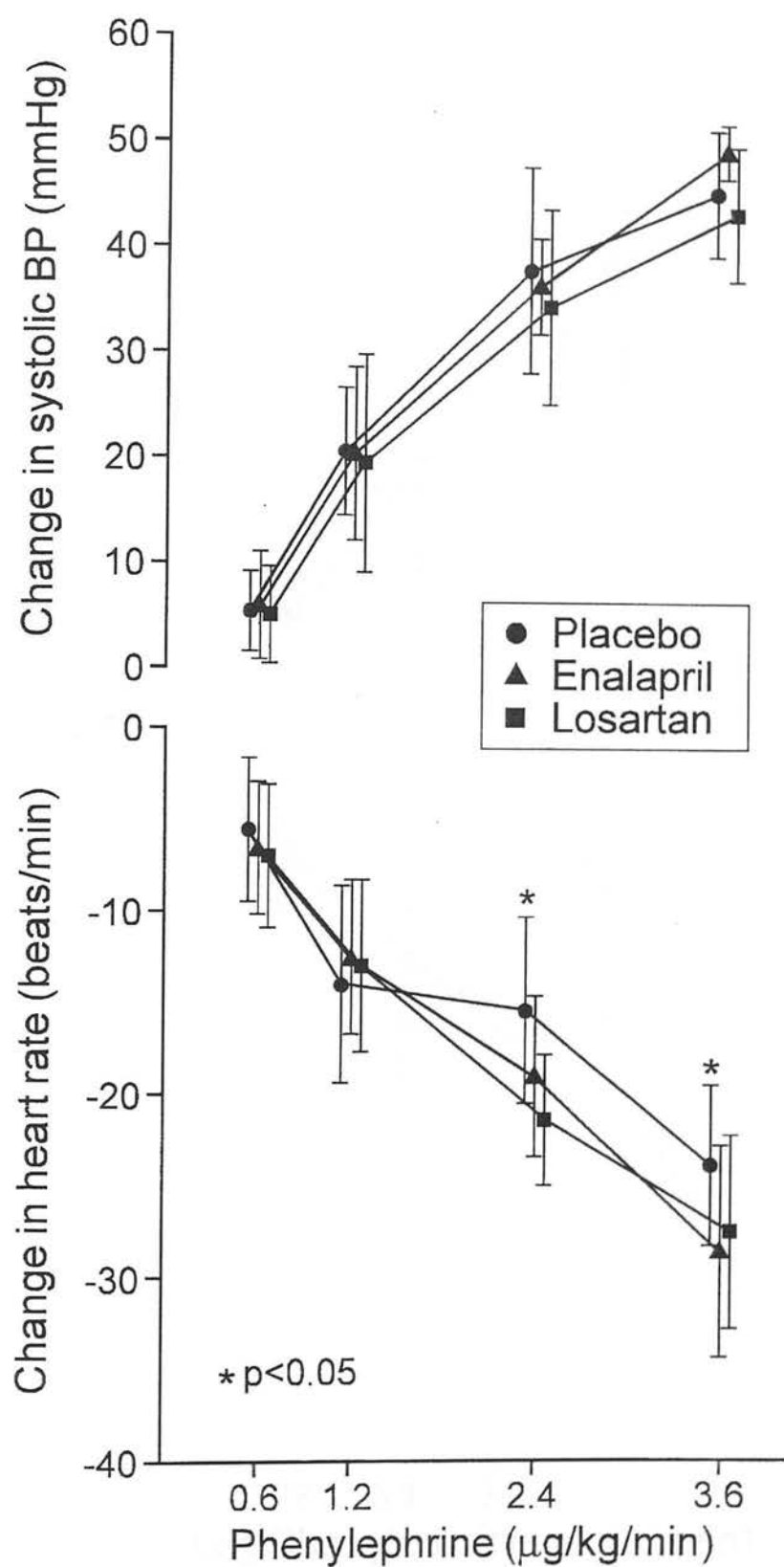
Dose PE(ug/kg/min)	Placebo	Enalapril	Losartan
<i>ΔSBP (mmHg)</i>			
0.6	5.3 \pm 3.8	5.9 \pm 5.1	5.0 \pm 4.6
1.2	20.6 \pm 6.1	20.4 \pm 8.3	19.5 \pm 10.4
2.4	38.1 \pm 9.8	36.6 \pm 4.5	34.6 \pm 9.3
3.6	45.6 \pm 6.0	49.6 \pm 2.6	43.6 \pm 6.4
<i>ΔHR (bt/min)</i>			
0.6	5.6 \pm 3.9	6.6 \pm 3.6	7.0 \pm 3.9
1.2	14.0 \pm 5.4	12.5 \pm 4.2	13.0 \pm 4.7
2.4	15.4 \pm 5.1	19.0 \pm 4.4*	21.4 \pm 3.6*
3.6	23.9 \pm 4.4	28.6 \pm 5.8†	27.5 \pm 5.3*
<i>ΔR-R Int (msec)</i>			
0.6	86 \pm 64.5	101.5 \pm 68	110 \pm 79
1.2	250 \pm 127	222 \pm 113	224 \pm 127
2.4	280 \pm 125	376 \pm 142*	421 \pm 149*
3.6	517 \pm 204	738 \pm 309*	644 \pm 269

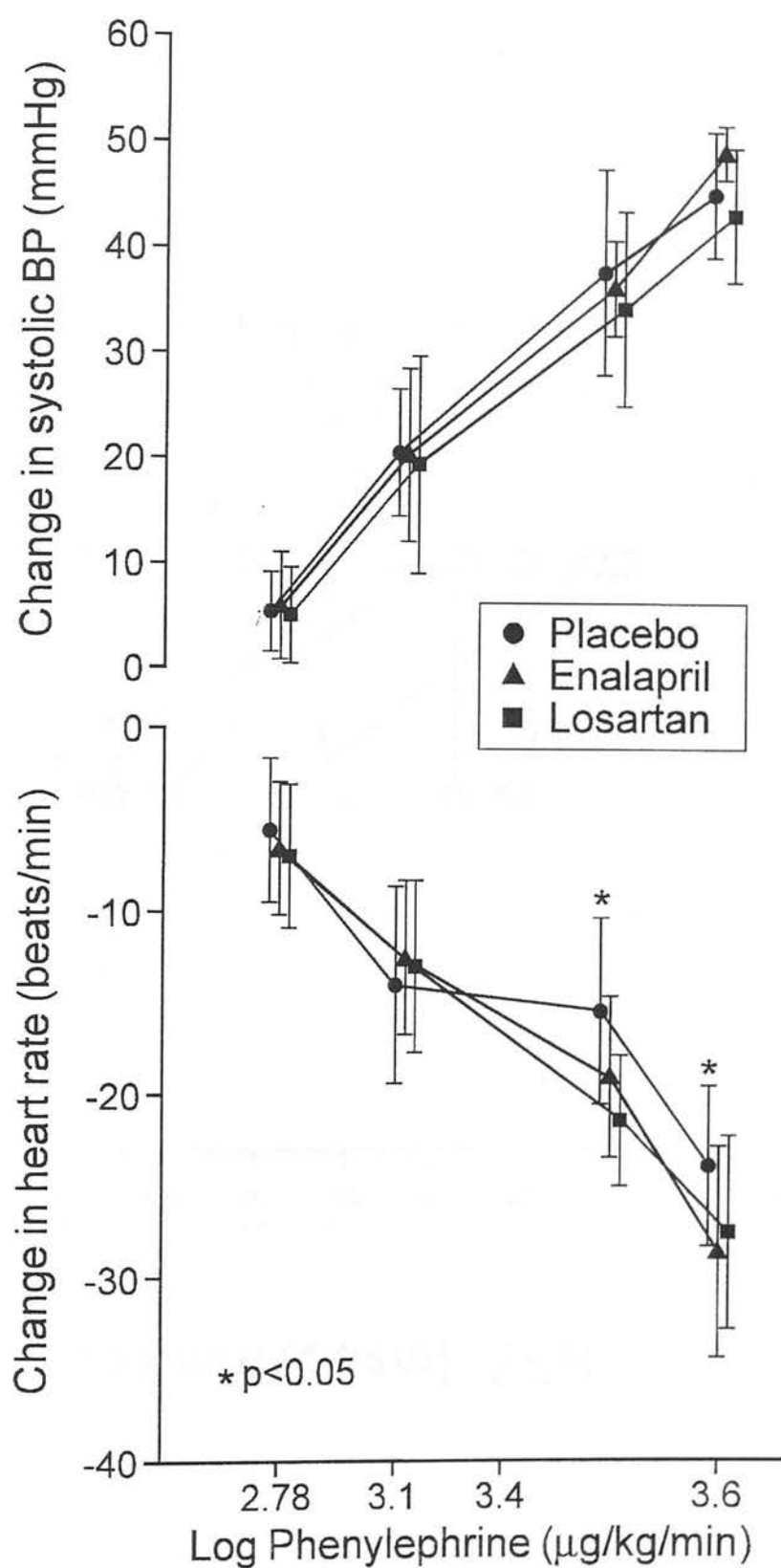
LEGENDS TO FIGURES

Fig 6.1: Change in Heart Rate (ΔHR) and Blood Pressure (ΔsBP) responses to incremental infusions of Phenylephrine. Values are mean \pm SD. * $p < 0.05$ compared with placebo.

Fig 6.2: Log Phenylephrine Dose-Response curve. Change in Heart Rate (ΔHR) and Blood Pressure (ΔsBP) responses to incremental infusions of Phenylephrine. Values are mean \pm SD. * $p < 0.05$ compared with placebo.

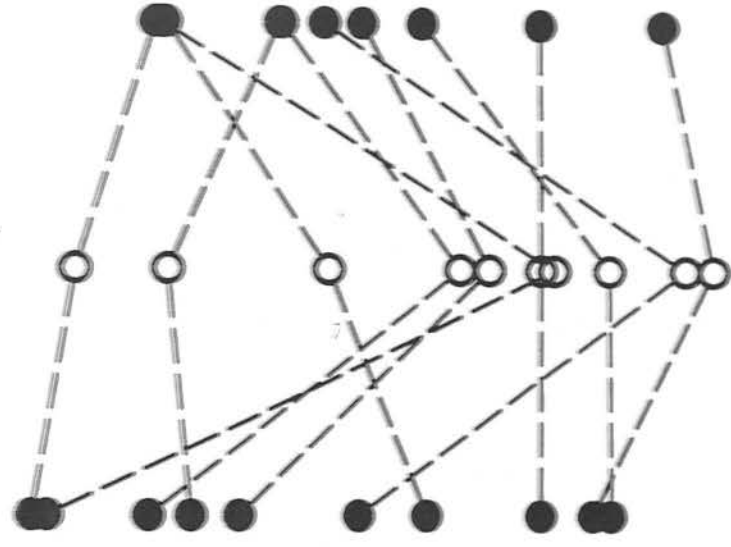
Fig 6.3: Individual Baroreflex Sensitivity (BRS) data. The individual BRS indices (slope of the linear regression line $\Delta RR / \Delta sBP$) in response to each treatment are displayed.





BRS (msec/mmHg)

20
18
16
14
12
10
8
6
4
2
0



enalapril placebo losartan

Treatment

6.4 DISCUSSION

In this study, the haemodynamic effects of a single dose of oral losartan potassium and a single dose of oral enalapril maleate were examined in salt-depleted normotensive subjects pre-treated with diuretics. Assessments were made 6 hrs after oral administration of the respective medications ie. at the time when the haemodynamic effects of the drugs are maximal [Brunner *et al* 1981, Ohtawa *et al* 1993]. The hypotensive effect of a single dose of 50mg losartan was comparable with that of 20 mg enalapril (systolic BP reduced by 8.4 mmHg [95% CI 4.2, 12.6] and 9.6 mmHg [95% CI 4.6, 14.6] respectively). In accordance with data from other studies [Brunner *et al* 1981, Ohtawa *et al* 1993, Gradman *et al* 1995, Ibsen *et al* 1983] resting blood pressure was significantly reduced by both drugs but resting heart rate was unaffected.

The absence of reflex tachycardia accompanying blood pressure reduction has been attributed to the parasympathetic activity of these drugs. The influence of Ang II on the cardiac vagal activity has certainly been well established in both animal studies [Potter 1982, Lumbers *et al* 1979] and human studies involving steady state infusions of Ang II [Mace *et al* 1985]. Although in disease states such as CHF, ACE inhibitors have been shown to enhance baroreceptor function [Osterziel *et al* 1990, Marakas *et al* 1995], the evidence for such a role for endogenous Ang II in healthy man has been conflicting. To date, a number of studies have been performed in both normotensive and hypertensive subjects. In sodium replete hypertensive subjects, captopril has been shown to cause displacement of the baroreceptor set-point but no modification of the BRS during activation by phenylephrine [Mancia *et al* 1982, Warren *et al* 1983]. However, hypertensive patients are known to have a blunted baroreflex function

[Warren *et al* 1983, Bristow *et al* 1969] which might confuse the picture. Hence, only studies in normotensive subjects will allow a true assessment of the effect of Ang II blockade *per se* on baroreceptor function. Amongst normotensive subjects, Ibsen *et al* [1983] found that enalapril improved BRS function whereas Guidicelli *et al* [1985] did not. In the only human study involving AT1 receptor antagonists, losartan had no effect on baroreflex sensitivity, although this was measured by the gain of the transfer function relating BP to pulse interval rather than by more standard techniques [Rongen *et al* 1998]. One of the possible reasons for the contradictory data in the literature may be related to the differences in the salt status and degree of endogenous Ang II activation in the different study populations. The latter two studies above [Rongen *et al* 1998, Guidicelli *et al* 1985] were carried out in normotensives with normal salt status whereas in the study by Ibsen *et al* [1983] which showed an improvement in baroreceptor function following ACE inhibitor therapy, the subjects were mildly sodium depleted. This observation may be important suggesting that the influence of endogenous Ang II on the autonomic and vascular tone may only become prominent during an activated RAAS state. For example, salt depletion may lead to activation of the RAAS and therefore allow for the influence of Ang II blockade on baroreceptor function to become detectable. It has been shown in a recent animal model that Ang II blockade improved the baroreflex response to a greater extent in rats fed with a sodium deficient diet compared with those on a high sodium diet [Xu and Brooks 1997]. The effects of raised endogenous Ang II is clinically relevant in a variety of cardiovascular diseases such as CHF and hypertension where the RAAS is activated and may even be further exacerbated by diuretic therapy. Recent data even suggest that the RAAS is activated in hypercholesterolaemic patients [Nickenig *et al*

1997] which may make the findings relevant to many disease groups.

The effects of raised endogenous Ang II on reflex baroreceptor function were assessed in this study. The subjects in the study were pretreated with frusemide to activate the RAAS. The reflex bradycardic response to phenylephrine was significantly increased following treatment with enalapril and losartan. In addition, the slopes of the $\Delta RR/\Delta sBP$ linear regression line were also significantly increased. These observations support our hypothesis that raised endogenous Ang II levels do contribute to baroreceptor dysfunction and that Ang II blockade, either by ACE inhibition or direct antagonism at the receptor level, would reverse it. These findings are also in agreement with observations from animal models where both losartan [Xu and Brooks 1997, Weinstock and Gorodetsky 1995] and enalapril [Weinstock and Gorodetsky 1995] have been shown to enhance baroreceptor sensitivity.

It is also interesting to note in this study that the effects of losartan were comparable to that of enalapril. Although minor differences between the two clearly could not be excluded, we were able to exclude large or significant differences. Direct Ang II receptor antagonists such as losartan lack the bradykinin potentiation seen with ACE inhibition. Although there are beneficial effects associated with bradykinins, the lack of bradykinin-related adverse effects makes AT1 receptor antagonists an attractive alternative to ACE inhibitors. The ELITE I trial [Pitt *et al* 1997] results intriguingly suggest that losartan may be better than ACE inhibitors in terms of reducing mortality and in particular sudden deaths in heart failure. Although baroreflex dysfunction is thought to be a key process leading to ventricular arrhythmias and sudden deaths, the present study did not however show any large differences in baroreceptor modulation between the two strategies. There may be

several explanations for the discrepancy in these findings. Firstly, the mechanisms for sudden death are multifactorial. Secondly, and perhaps most importantly, the number of deaths in the ELITE I trial was small (49 deaths) as it was not designed primarily as a mortality trial and the results may have occurred by chance. The results of the more recent ELITE II Study [Pitt *et al* 2000] would certainly support this notion. Unlike ELITE I, the latter was a much larger trial (four times as many patients-3152 patients-and ten times more events) with all-cause mortality as its primary end-point. Over a mean follow-up period of 555 days, losartan did not appear to concur any survival advantage compared to captopril in NYHA II-IV heart failure patients. The inability of ELITE II to confirm a survival advantage of losartan over captopril highlights the need for caution in the interpretation of trial results where the event numbers were small which may be influenced by the play of chance.

Finally, there are inevitably, some limitations to the study, which are worth discussing. As in the study carried out in *Chapter 5*, infusions rather than boluses of pressor stimuli were used in this study, allowing for some baroreceptor “resetting” to occur and hence reducing or dampening any change in baroreceptor response. This may mean that potentially small differences in baroreceptor response between the two treatments for instance, may have gone undetected. The limitations associated with the infusion method has been previously described in *Chapters 2* and *5*. However as each of our readings were taken in triplicate, random measurement error should be minimised and any potential differences between the two treatments that may have gone undetected by the study is likely to be small.

Despite these limitations, our data clearly indicates that blocking endogenous Ang II in man improves baroreceptor function. Both strategies, AT1 receptor

antagonism and ACE inhibition appear to be equally effective in restoring baroreceptor function in salt-depleted normotensive subjects. This is clinically relevant and suggest that AT1 blockers may be an attractive alternative to ACE inhibitors in cardiovascular diseases such as CHF where the RAAS is activated. Clearly, the neurohormonal activation and haemodynamic responses are much more complicated in the CHF patient, and our observations need to be confirmed in this cohort of patients. The long term effects of these treatments have been evaluated in the ELITE II mortality trial. Although no mortality benefit was demonstrated, losartan did appear to be better tolerated than captopril in the ELITE II trial. This may be an equally important observation as an agent that is as effective as ACE inhibitors but better tolerated would make a significant impact on the lives of CHF patients. However it must be noted that ELITE II was designed as a superiority trial; it was not designed to address equivalence and certainly the data was insufficient to claim that the two treatments were equally effective.

For the moment ACE inhibitors remain the drug of choice although AT1 receptor antagonists, like aldosterone antagonists, may offer us an attractive therapeutic strategy to overcome the neurohormonal re-activation seen with chronic ACE inhibition. The principal results of the Val HeFT (Valsartan in Heart Failure Trial) were recently presented at the American Heart Association Scientific Sessions (New Orleans, Nov 2000). In this placebo-controlled trial where 5010 NYHA Class II-IV patients were randomised, the hypothesis that adding valsartan to conventional therapy (including ACE inhibitors and beta-blockers) would improve clinical outcomes was tested. Although Valsartan did not reduce all-cause mortality, CHF hospitalisations was significantly reduced (27% reduction, $p=0.000001$). Important

issues regarding its efficacy have been highlighted in the subgroup analyses, which raises the possibility of drug interactions between valsartan and ACE inhibitors/beta-blockers. Therefore, clinicians should at least await the full publication of the ValHeFT results and seek confirmation from other ongoing randomised trials (such as CHARM for candesartan) before offering their CHF patients this class of drugs as a more tolerable and equally effective alternative to ACE inhibitors.

CHAPTER SEVEN

DETERMINANTS OF QT DISPERSION IN MAN:

THE EFFECTS OF ALDOSTERONE,

AFTERLOAD AND VAGAL BLOCKADE

7.1 INTRODUCTION:

In *Chapter 3*, spironolactone was found to improve QT dispersion in heart failure patients. QT dispersion is however likely to be due to a multitude of different factors. In order to understand the mechanism by which spironolactone improves QT dispersion, we need to understand more about the various factors which determine an individual's QT dispersion. In the present chapter, two studies examining some of these potential mechanisms are described.

One factor which may be important is cardiac afterload. Many factors determine afterload but aldosterone may be a contributor since it has receptors in vascular tissue and has been shown to increase vascular tone [Weber and Purdy 1962], increase AT-1 receptor density [Ullian *et al* 1992] and potentiate Ang II-induced hypertrophy of vascular smooth muscle cells [Hatakeyama *et al* 1994]. The first study was therefore designed to assess if changes in cardiac afterload *per se* would affect QT interval dispersion. In experimental models, an acute increase in cardiac afterload has been shown to be arrhythmogenic [Han *et al* 1990, Sideris *et al* 1995]. Therefore part of the beneficial effects on QT dispersion seen with ACE inhibitors and spironolactone may be attributed to their ability to reduce afterload. Alterations in cardiac loading may affect myocardial electrophysiology by the mechanism of mechano-electrical feedback, or contraction-excitation coupling [Lab 1982]. In experimental studies, an increase in cardiac loading with subsequent stretching of the ventricular myocardium causes shortening of the action potential duration [Lab 1982, Taggart *et al* 1992], and this in turn, may result in an altered dispersion of action potential repolarisation in the ventricle [Dean and Lab 1990]. Until now, there has been little evidence for this occurring in man *in vivo*.

We therefore examined the effects of an acute phenylephrine-induced increase in afterload on QTd in man. As phenylephrine is known to alter heart rate significantly (via a reflex vagal-response), the study was carried out, with and without the presence of atropine, given to inhibit the reflex parasympathetic activity. We also examined whether atropine *per se* would alter the QT_{max} interval and QT dispersion in this study.

The effects of an acute intravenous infusion of aldosterone itself on QT dispersion in man were explored in the second study. The detection of mineralocorticoid receptors in the heart [Agarwal and Philippe 1979] suggest that aldosterone may have direct effects on the myocardium. Certainly in an animal model by Arora and Somani [1962], the direct administration of aldosterone infusion into coronary ligated dogs was associated with the generation of ventricular arrhythmias.

7.2 METHODS:

In both studies, subjects were asked to refrain from alcohol, caffeine and cigarettes for 24 hrs and to fast for 2 hrs before each study day. Subjects rested quietly in the supine position throughout the study.

7.2.1 Study 1

Subjects And Protocol

10 normal male volunteers [mean age (SD) 25 (4.5)] were studied. One had to be withdrawn from the study as he was not able to tolerate the phenylephrine infusion (his arterial SBP increased by >50mmHg even at the lowest dose administered). Subjects were studied on 2 separate days, at least 72 hours apart, in a placebo-

controlled, randomized, single-blinded, cross-over fashion. A single 18G intravenous cannula was inserted into a right forearm vein of each subject. After 30 minutes of bedrest, baseline values of blood pressure (BP) and heart rate (HR) were measured non-invasively in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, USA) with the cuff being placed around the subjects left arm. Baseline 12-lead electrocardiograms (ECG) were also obtained.

Following this, a continuous infusion of either phenylephrine (Knoll Pharma, UK) or placebo (5% Dextrose) in similar volumes, was commenced intravenously. Up to 6 incremental stepwise 10 minutes infusions (0.2-3.6 ug/kg/min) were administered using an infusion pump (IMED, San Diego, CA). The infusion was stopped when a 35-40 mmHg rise in systolic BP had been achieved with the active treatment. Infusions of the placebo were carried out in similar volumes and incremental steps as the active treatment. BP, HR and electrocardiogram recordings were obtained in triplicate between 8 and 10 minutes after each infusion dose. After completion of these measurements, the infusion was discontinued and resting BP and HR were allowed to return to baseline values for 30 minutes.

Atropine 0.03mg/kg was then administered by slow iv injection over 1 minute. Once heart rate and blood pressure had restabilised following full vagal blockade, a repeat baseline 12-lead ECG was obtained and the infusion of phenylephrine (or placebo) was restarted and the study repeated again as described above. Forty five minutes after the injection of atropine, a further 0.6mg atropine was given intravenously to ensure continued vagal blockade. At the end of the study resting blood pressure and heart rate were allowed to return to normal.

7.2.2 Study 2

Subjects And Protocol

10 normal male volunteers [mean age (SD) 23 (7)] were studied on 2 separate days, at least 72 hours apart, in a placebo-controlled, randomized, double-blind, cross-over fashion. Two 18G intravenous cannulae were inserted into forearm veins, one in the right arm for blood sampling and one in the left arm for infusion of either aldosterone or placebo. After 45 minutes of bedrest, baseline values of blood pressure (BP) and heart rate (HR) were measured non-invasively in triplicate using a semi-automatic sphygmomanometer with the cuff being placed around the subjects left arm. 12 lead electrocardiograms and venous blood (15mls) for baseline aldosterone and angiotensin II assays were also obtained.

Following this, a continuous infusion of either placebo (5% Dextrose) or d-Aldosterone (Tayside Pharmaceuticals) in similar volumes was commenced in the left arm. The d-aldosterone was infused at a rate of 12pmol/kg/min. After 45 minutes into the infusion, further triplicate recordings of blood pressure, heart rate and continuous 12-lead ECGs were obtained along with blood samples for aldosterone and angiotensin II assays.

QT Interval And Dispersion Analysis

In both studies, QT interval analysis was carried out blindly on electrocardiograms (with simultaneous 12-lead acquisition) as detailed in *Chapter 2: Methods*. In addition to Bazett's formula ($QT_c = QT/RR^{1/2}$) for heart-rate correction,

the QT intervals were also corrected using the Morrison and Hodges [Hodges *et al* 1983] linear correction formula [$QT_c = QT + 1.75 (HR - 60)$] in *Study 1*.

Aldosterone And Angiotensin II Assays

As described in *Chapter 2*.

Statistical Analysis

All data were analyzed using the Statgraphics software package (STSC Software Publishing Group, Rockville, MD, USA) as detailed in *Chapter 2: Methods*. The relationships between variables in study were studied by Pearson correlation and linear regression analyses. Differences between treatment groups were analyzed using the paired Student *t* test.

7.3 RESULTS:

7.3.1 Study 1

Baseline Measurements (See Table 7.1)

Resting blood pressure, heart rate and ECG parameters were similar at the start of both study visits (*Table 7.1*). Following atropine, baseline HR increased significantly but resting blood pressure was unchanged. This was paralleled by a significant reduction in QT_{max} and an increase in the Bazett-corrected QT_{cmax} . The linearly-corrected QT_{cmax} was non-significantly increased. Atropine however did not appear to have any significant effect on either QT_d or QT_{cd} .

Correlation between alterations in blood pressure and QT dispersion

Phenylephrine alone: Phenylephrine given in incremental doses caused a gradual rise in blood pressure paralleled by a significant fall in reflex heart rate. Both QT_{max} and the linearly-corrected QT_{cmax} significantly increased but the Bazett-corrected QT_{cmax} was not significantly affected by the changes in blood pressure induced by phenylephrine (see *Table 7.2*). The relationships between changes in blood pressure and QT interval and dispersion indices in response to phenylephrine are displayed in *Fig 7.1-7.5* and *Table 7.3*. It is clear that phenylephrine causes a significant increase in QTd and QTcd and that this occurs in the presence or absence of atropine.

Changes in QTd (ΔQTd) and QTcd ($\Delta QTcd$) appear to be significantly correlated with both changes in systolic and diastolic BP (ΔSBP and ΔDBP) in a linear fashion. The relationships between the variables, expressed as the slope of the linear regression line (eg $\Delta QTd / \Delta SBP$), and their respective p -values and correlation coefficients for each individual are displayed in *Table 7.3*.

Similar linear regression analysis made on the pooled data (pooled correlation coefficients and p values are displayed in *Fig 7.2-7.4* respectively) yielded comparable results confirming the above observations.

Phenylephrine and Atropine: Following pre-treatment with atropine, phenylephrine had no significant effect on either heart rate, QT_{max} or QT_{cmax} (*Table 7.2*). The previously demonstrated relationship between the dispersion indices and blood pressure was still present and was unaffected by atropine pre-treatment. There were no significant differences in the slope, $\Delta QTd / \Delta SBP$ before and after atropine [Mean difference -0.003 (95% CI -0.558 to 0.552) msec/mmHg; $p=0.9$]. Similarly,

atropine did not affect $\Delta QTd/\Delta DBP$ [Mean diff -0.271 (95% CI -1.060 to 0.517) msec/mmHg; $p = 0.45$), $\Delta QTcd/\Delta SBP$ [Mean diff -0.404 msec/mmHg (95% CI -1.066 to 0.258) msec/mmHg; $p = 0.2$] or $\Delta QTcd/\Delta DBP$ [Mean diff -0.383 (95% CI -1.214 to 0.449) msec/mmHg; $p = 0.32$].

7.3.2 Study 2 (See Table 7.4-7.5)

No significant differences in baseline measurements were seen between placebo and aldosterone infusion days. A significant rise in plasma aldosterone levels was noted after 45 mins of aldosterone infusion compared to placebo. There were no significant differences in Ang II levels following aldosterone infusion compared to placebo. All QT interval and dispersion indices were not significantly affected by the aldosterone infusion compared to placebo.

Table 7.1: Baseline haemodynamic and electrocardiographic measurements with:

a) Placebo infusion. Expressed as Mean \pm SD. Statistical significance ($p < 0.05$) refers to comparison with pre-infusion baseline values.

	<i>Pre-infusion (Start)</i>	<i>Post -atropine*</i>	<i>P value</i>
SBP (mmHg)	114 \pm 10	116 \pm 11	NS
DBP (mmHg)	64 \pm 12	66 \pm 9	NS
HR (bt/min)	66 \pm 12	109 \pm 10	<0.001
QTmax (msec)	401.5 \pm 33	347 \pm 19	<0.001
QTcmax (<i>linear corr.</i>)	416 \pm 19	421 \pm 17	NS
QTcmax (<i>Bazett's corr.</i>)	412 \pm 14	448 \pm 15	<0.001
QTd (msec)	44.9 \pm 14.7	45.8 \pm 5.8	NS
QTcd (<i>linear corr.</i>)	44.9 \pm 14.7	45.8 \pm 5.8	NS
QTcd (<i>Bazett's corr.</i>)	47.3 \pm 15.4	54.4 \pm 9.7	NS

b) Phenylephrine infusion

	<i>Pre-infusion (Start)</i>	<i>Post -atropine*</i>	<i>P value</i>
SBP (mmHg)	116 \pm 11	121 \pm 13.5	NS
DBP (mmHg)	63 \pm 12	68 \pm 11.5	NS
HR (bt/min)	58 \pm 11	104 \pm 13	<0.001
QTmax (msec)	418 \pm 27	345 \pm 27	<0.001
QTcmax (<i>linear corr.</i>)	415 \pm 20	422 \pm 18	NS
QTcmax (<i>Bazett's corr.</i>)	410 \pm 23	455 \pm 24	0.002
QTd (msec)	45.8 \pm 10.7	46.1 \pm 12.0	NS
QTcd (<i>linear corr.</i>)	45.8 \pm 10.7	46.1 \pm 12.0	NS
QTcd (<i>Bazett's corr.</i>)	46.3 \pm 13.2	55.1 \pm 11.9	NS

* denotes resting values following atropinisation prior to re-commencement of phenylephrine (or placebo) infusion (*see text*).

Table 7.2: Effects of Phenylephrine (\pm Atropine) on Heart Rate and QT Intervals.

Measurements taken at baseline and after phenylephrine infusion, with and without prior atropinisation (*Phe-Atr* and *Phe*, respectively)

	<i>Baseline (no atropine)</i>	<i>Phe</i>	<i>P value</i>
HR	58 \pm 11	42 \pm 4	0.0004
QTmax	418 \pm 27	460 \pm 26.5	0.0003
QTcmax (<i>linear corr.</i>)	415 \pm 20	429 \pm 25	0.017
QTcmax (<i>Bazett's corr.</i>)	410 \pm 22.5	400 \pm 28	NS
QTmin	373 \pm 25	396 \pm 24	0.001
QTcmin (<i>linear corr.</i>)	369 \pm 20	364 \pm 20	NS
QTcmin (<i>Bazett's corr.</i>)	364 \pm 24	332 \pm 25	0.001
	<i>Baseline (with atropine)</i>	<i>Phe-Atr</i>	
HR	104 \pm 13	106 \pm 13	NS
QTmax	345 \pm 27	348 \pm 25	NS
QTcmax (<i>linear corr.</i>)	422 \pm 18	429 \pm 13	NS
QTcmax (<i>Bazett's corr.</i>)	455 \pm 24	465 \pm 14	NS
QTmin	299 \pm 24	285 \pm 17	0.038
QTcmin (<i>linear corr.</i>)	376 \pm 16	365 \pm 14	NS
QTcmin (<i>Bazett's corr.</i>)	398 \pm 16	381 \pm 12	0.02

Table 7.3: Effect of Changes in Blood Pressure on QT Dispersion: Individual data.

Relationship between changes in SBP (Δ SBP) and DBP (Δ DBP), and QTd are expressed in terms of the linear regression slope (Δ QTd/ Δ BP) for each individual, in response to phenylephrine (\pm atropine). r (in brackets) denotes the linear correlation coefficient. $P < 0.05$ denotes a statistically significant relationship between the two variables.

Patient.no.	Δ QTd / Δ SBP (msec/mmHg)	Δ QTd / Δ DBP (msec/mmHg)	Δ QTcd / Δ SBP (msec/mmHg)	Δ QTcd / Δ DBP (msec/mmHg)
	Phe (r)	Phe-Atr (r)	Phe (r)	Phe-Atr (r)
1	0.610 (0.61)	0.685 (0.98)	0.788 (0.60)	0.952 (0.98)
2	0.891 (0.98)	-0.190 (0.98)	1.700 (0.96)	-0.395 (0.93)
3	1.130 (0.84)	0.640 (0.68)	1.821 (0.88)	0.726 (0.40)
4	0.460 (0.98)	2.071 (0.95)	1.022 (0.99)	1.538 (0.90)
5	0.512 (0.81)	0.320 (0.64)	0.520 (0.80)	0.520 (0.70)
6	0.490 (0.81)	0.780 (0.75)	0.819 (0.80)	0.461 (0.20)
7	0.311 (0.86)	0.395 (0.96)	0.987 (0.95)	0.176 (0.99)
8	0.550 (0.74)	0.381 (0.99)	0.806 (0.82)	0.516 (0.96)
9	0.625 (0.98)	0.525 (0.98)	0.706 (0.99)	2.234 (0.62)
Mean	0.619	0.623	1.019	0.748
95% CI	(0.429- 0.809)	(0.152- 1.093)	(0.676- 1.362)	(0.159- 1.336)
P value	<0.0001	0.016	0.0001	0.019
			0.0001	0.015
			0.424	0.828
			(0.279- 0.570)	(0.211- 1.445)
			0.706	0.466- 0.946)
				(0.329- 1.849)
				0.011
				3.024 (0.62)

Fig 7.1 Individual data: Changes in QT dispersion (ΔQTd) in response to changes in systolic blood pressure (ΔSBP) following *Phe* infusion alone (top) and *Phe* + *Atropine* (bottom).

Fig 7.2 Pooled Scatterplot: Relationship between changes in QT dispersion (ΔQTd) and changes in systolic BP (ΔSBP) [*top*] and diastolic BP (ΔDBP) [*bottom*] in response to *Phe* \pm *Atropine*. The correlation coefficient, *r* and *p* value for each linear relationship are displayed.

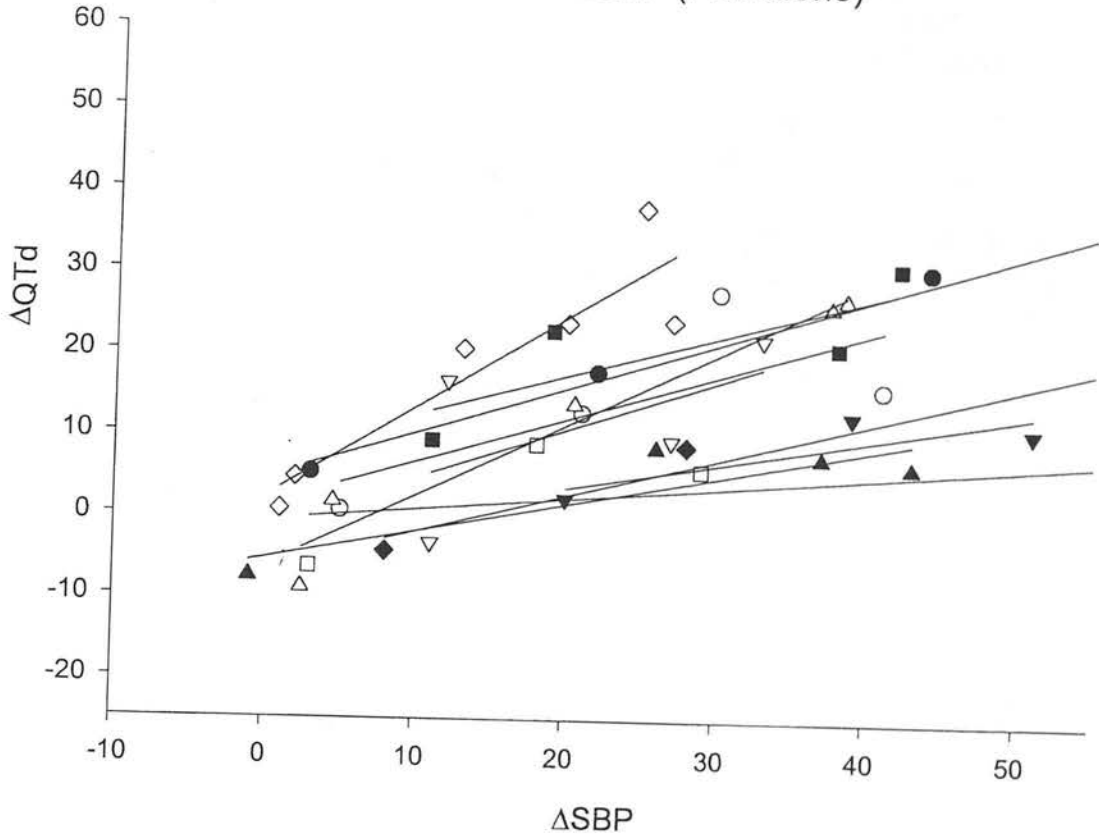
Fig 7.3 Pooled Scatterplot: Relationship between changes in QTc dispersion ($\Delta QTcd$) and changes in systolic BP (ΔSBP) [*top*] and diastolic BP (ΔDBP) [*bottom*] in response to *Phe* \pm *Atropine*. The correlation coefficient, *r* and *p* value for each linear relationship are displayed.

Fig 7.4 Pooled Scatterplot: Relationship between changes in Bazett-corrected QT_{cmax} (ΔQT_{cmax}) and changes in systolic BP (ΔSBP) [*top*] and diastolic BP (ΔDBP) [*bottom*] in response to *Phe* \pm *Atropine*. The correlation coefficient, *r* and *p* value for each linear relationship are displayed.

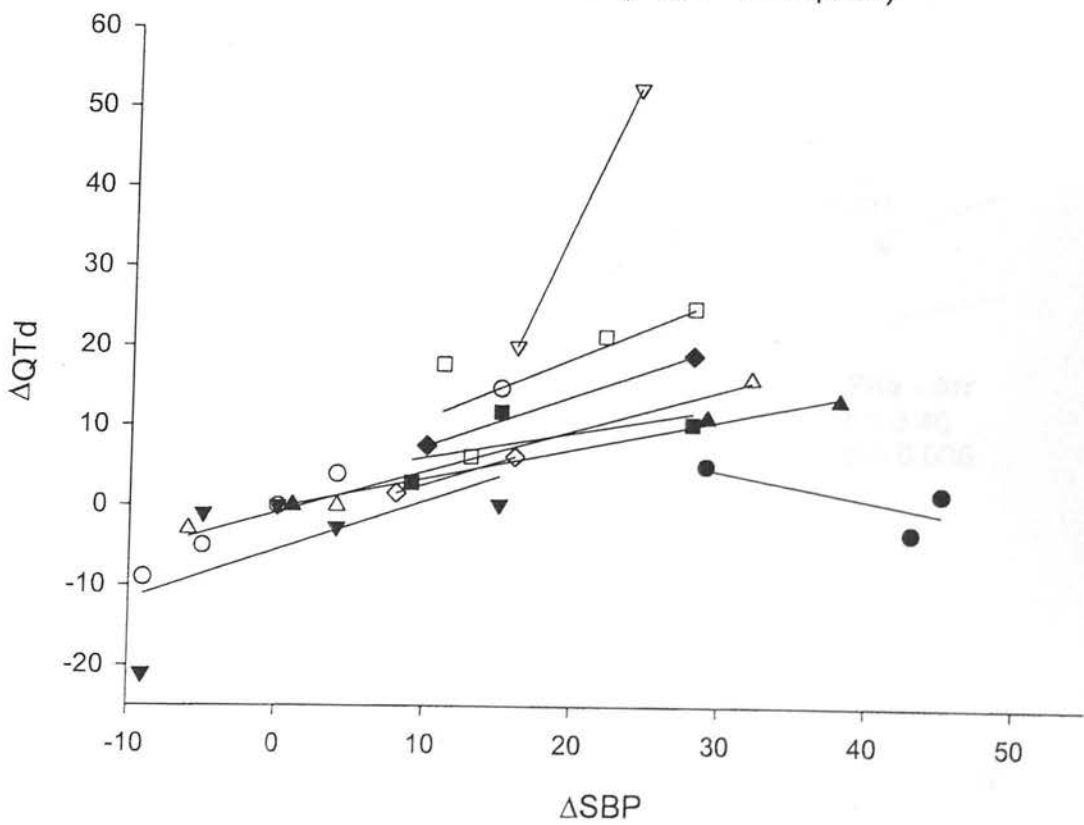
Fig 7.5 Pooled Scatterplot: Relationship between changes in QT_{min} [*top*] and Bazett-corrected QT_{cmin} (ΔQT_{cmin}) [*bottom*] and changes in systolic BP (ΔSBP) in response to *Phe* \pm *Atropine*. The correlation coefficient, *r* and *p* value for each linear relationship are displayed.

Fig 1

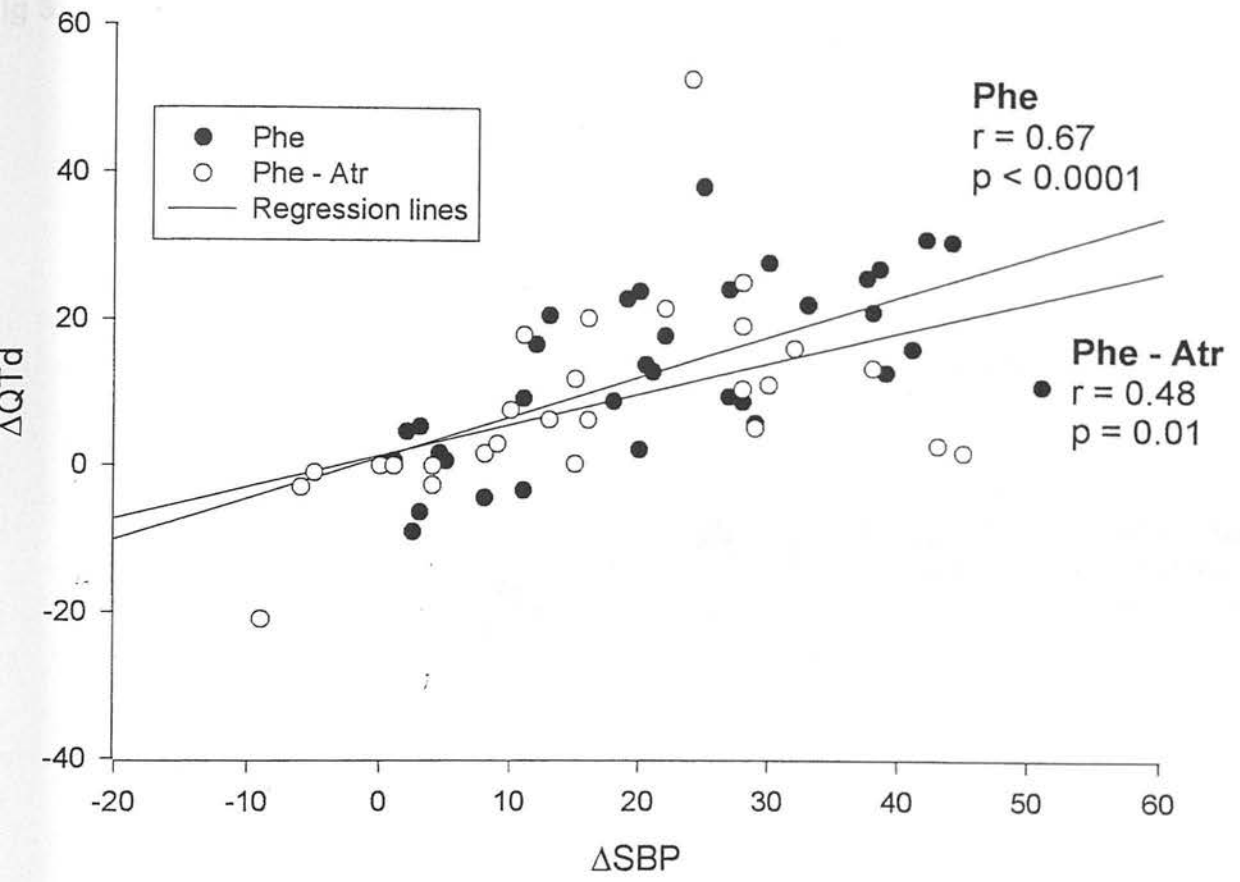
$\Delta Q T d$ vs $\Delta S B P$ (Phe alone)



$\Delta Q T d$ vs $\Delta S B P$ (Phe + Atropine)



$\Delta Q T d$ vs $\Delta S B P$



$\Delta Q T d$ vs $\Delta D B P$

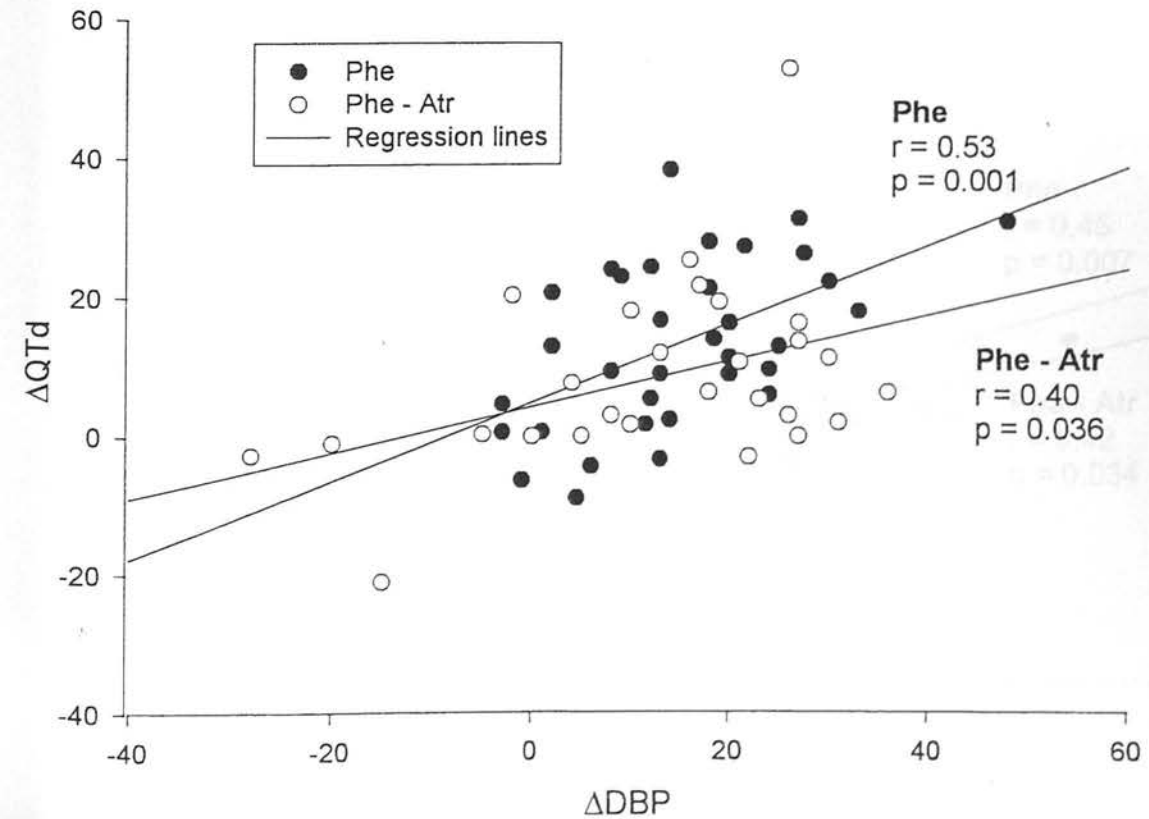
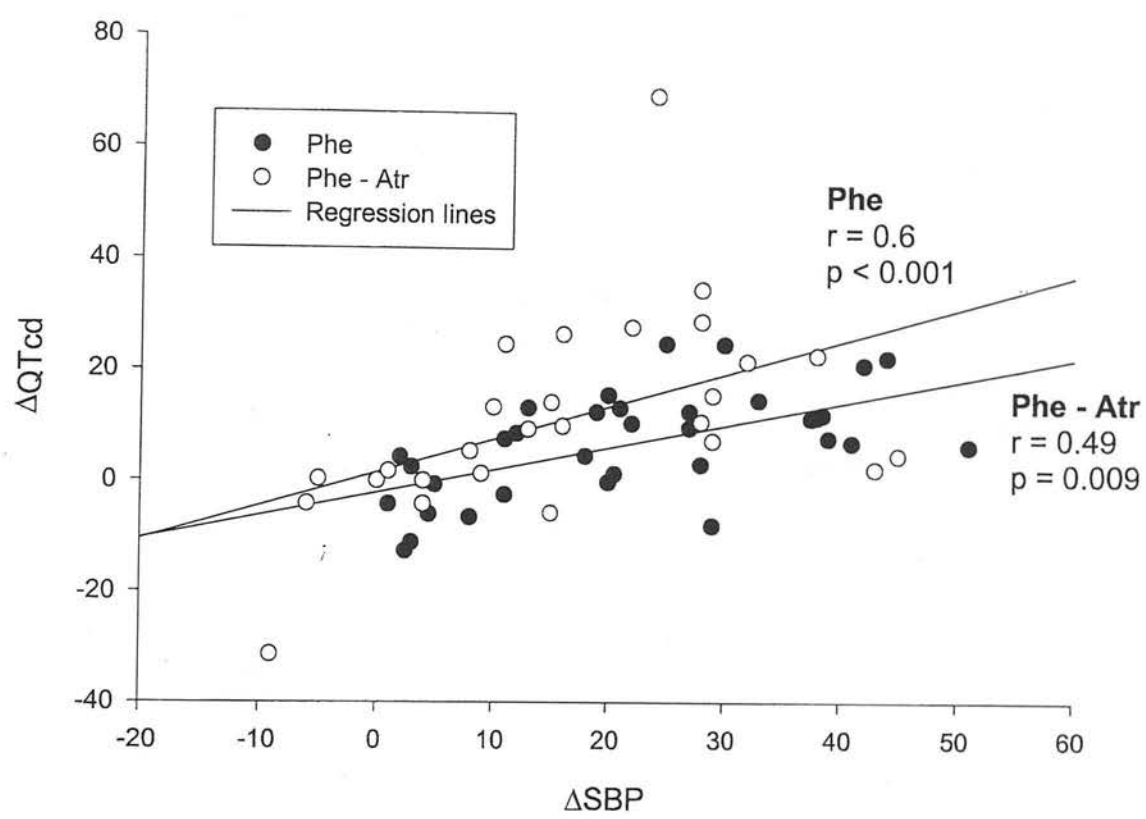


Fig 3

$\Delta Q T c d$ vs $\Delta S B P$



$\Delta Q T c d$ vs $\Delta D B P$

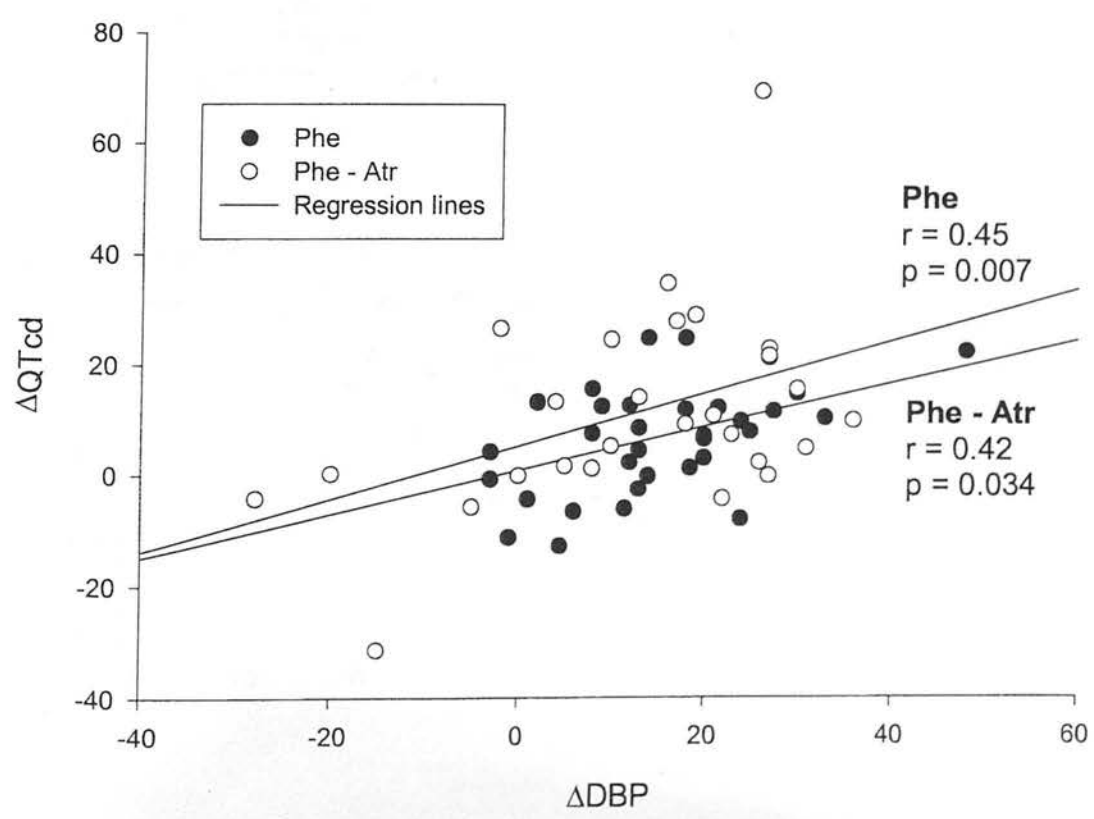
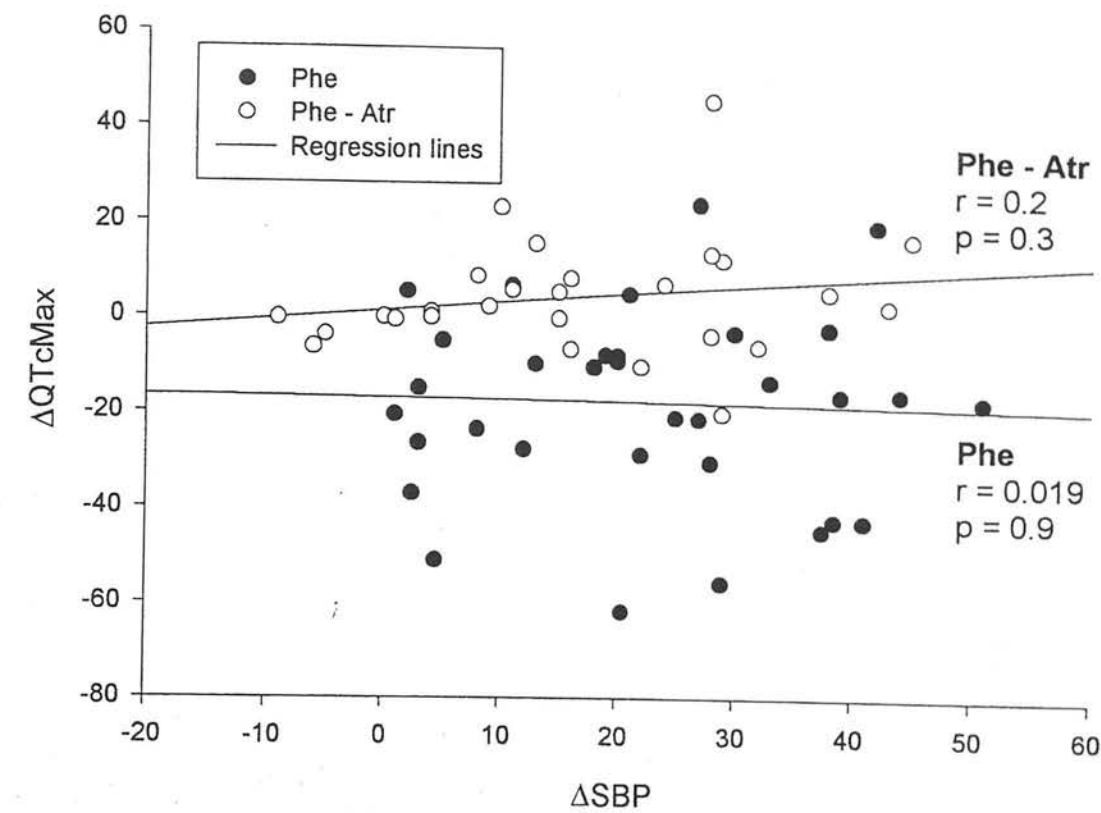


Fig 4

Δ QTcMax (Bazett) vs Δ SBP



Δ QTcMax (Bazett) vs Δ DBP

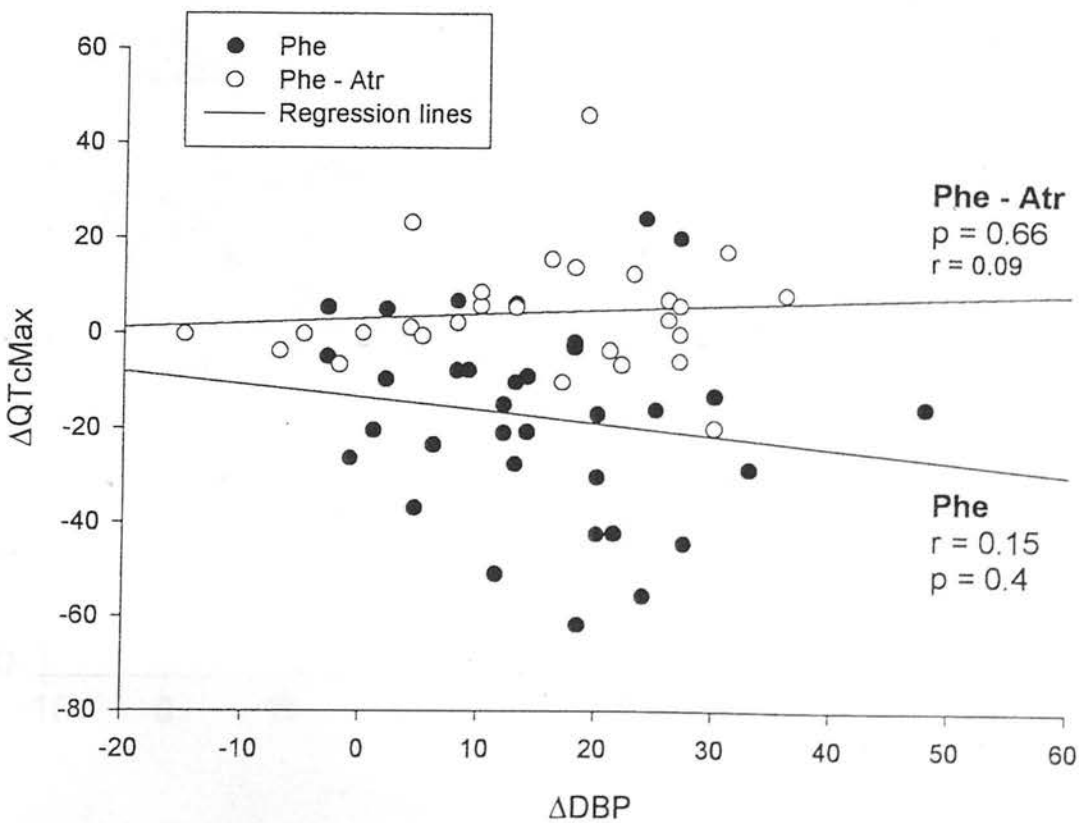
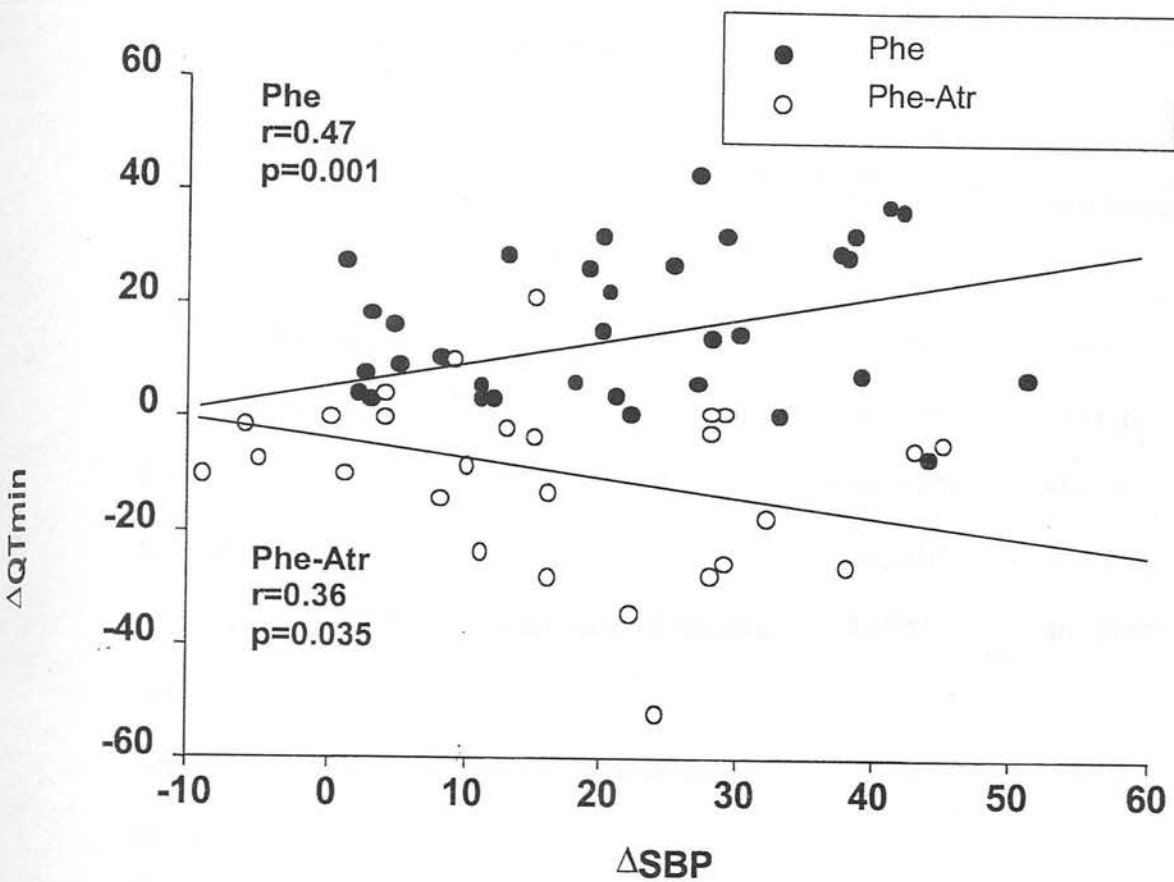


Fig 5

Δ QTmin vs Δ SBP



Δ QTcmin (Bazett) vs Δ SBP

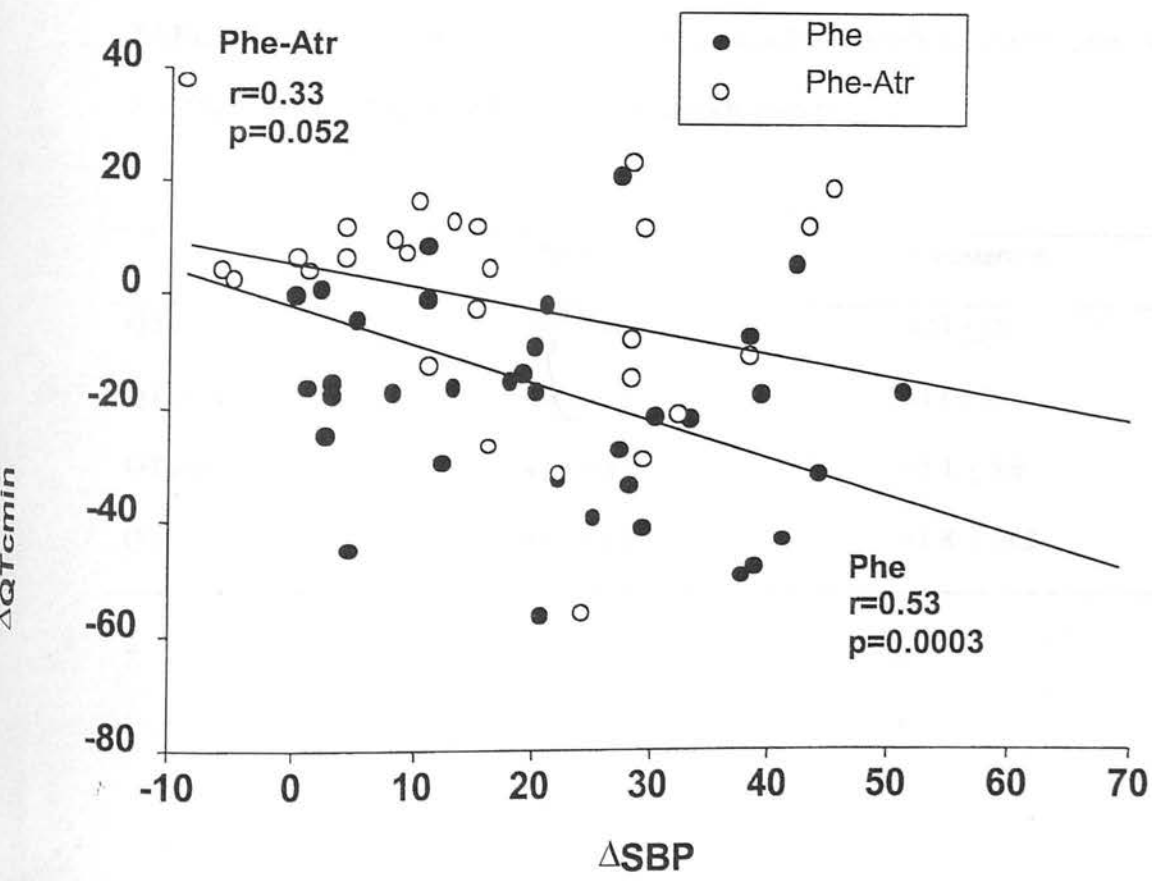


TABLE 7.4: Baseline values (Study 2). Results are expressed as Mean \pm SD. Statistical significance : *P<0.05 compared with placebo.

	Placebo	Aldosterone
Heart rate (beats/min)	62 (7)	64 (8)
Systolic blood pressure (mmHg)	117 (10)	113 (6)
R-R Interval (msec)	1004 (167)	989 (175)
Aldosterone- baseline levels (pg/ml)	104 (101)	113 (90)
Aldosterone level- 45 mins after start of infusion (pg/ml)	89 (78)	467 (90)*
Angiotensin II levels – 45 mins after start of infusion (pg/ml)	17 (8)	13.5 (7)

TABLE 7.5: ECG measurements after 45 mins infusion: Results are expressed as Mean \pm SD. Statistical significance : *P<0.05 compared with placebo.

	Placebo	Aldosterone
QT _{max}	409 \pm 23	420 \pm 30
QT _{cmax}	414 \pm 18	417 \pm 24
QT _{disp}	42.4 \pm 11.7	45.1 \pm 9.6
QT _{cdisp}	44.8 \pm 10.5	41.8 \pm 10.2

7.4 DISCUSSION:

Mechanisms in which aldosterone may contribute towards dispersion of the QT interval are explored in this chapter. An acute aldosterone infusion did not appear to have any significant direct effects on QT dispersion in man. However in *Study 1*, which was designed to examine the effects of increasing cardiac afterload on QT dispersion, a significant positive correlation between changes in blood pressure and changes in all QT dispersion indices (QTd, Bazett-corrected QTcd and linear-corrected QTcd) was found. Importantly, the atropine data showed that this relationship between all QT dispersion indices and afterload was independent of heart rate changes or reflex vagal activity. These observations also suggest that reflex vagal activity does not influence QT dispersion.

Phenylephrine is a vasopressor, acting principally on the arterial bed, causing vasoconstriction, an increase in peripheral resistance and a corresponding rise in blood pressure. This increase in afterload is accompanied by a fall in heart rate, resulting from reflex activation of the parasympathetic nervous system. Therefore, to exclude the potentially confounding effects of reflex vagal activity, the study was also carried out in the presence of atropine. Pre-treatment with atropine not only enabled us to assess the contributory role of vagal blockade on QT dispersion, but also allowed us to control for heart rate (which remained unchanged) during the combined infusion of phenylephrine and atropine.

Due to the varying HRs, the effects of phenylephrine on Bazett's rate corrected QT intervals *were significantly different* from uncorrected values in our study (*Tables 7.1 and 7.2*). As one can clearly see, the QTmax values were not only significantly different between corrected and uncorrected values but they moved

numerically in *opposite* directions, as a result of over-correction with the Bazett's formula [Browne *et al* 1983, Ahnve and Vallin 1982].

However, despite this, *the relationship between changes in QT dispersion and changes in BP was unaffected by HR (Fig 7.2-7.3)*. Although it appears that acute cardiac afterload increases QT dispersion, there does not appear to be any significant correlation with QT_{cmax} (Fig 7.4). The impact of these major haemodynamic perturbations appears to be mainly on the QT_{cmin} (Table 7.2, Fig 7.5). The reduction in QT_{cmin} with afterload is consistent with experimental data where stretching of the ventricular myocardium has been shown to shorten action potential duration [Lab 1982, Taggart *et al* 1992, Dean and Lab 1990].

7.4.1 Afterload And QT Dispersion

Our findings are consistent with the results of a recent study by Sun *et al* [1998] who found a 22% increase (although non-significant) in dispersion of the QT interval following phenylephrine infusion in normal subjects. It is well established from experimental models that alterations in cardiac afterload may affect the action potential duration through mechano-electrical feedback [Lab 1982]. From the law of Laplace, an increase in afterload should result in an increase in wall stress. Stretching of the ventricular myocardium has been shown to shorten the action potential duration in a number of studies [Lab 1982, Taggart *et al* 1992, Dean and Lab 1990]. Importantly however, these changes in effective refractory period may not be homogenous throughout the myocardium. Dean and Lab [1990] found that in an *in situ* pig heart model for instance, there was a greater change in refractory period occurring at the apex compared to the base in response to an increase in load by aortic

clamping. These animal observations of increased regional dispersion of repolarisation across the left ventricle agree with the results of our study in man.

The dispersion of ventricular action potential provide a substrate for re-entrant circuits and the development of malignant arrhythmias [Han and Moe 1964, Kuo *et al* 1983]. Hence, the effects of afterload on QT dispersion have important clinical implications. A number of previous studies have shown that an increase in afterload is arrhythmogenic [Hansen *et al* 1990, Sideris *et al* 1995]. Furthermore, patients with conditions associated with a raised afterload (eg. chronic heart failure, aortic stenosis, hypertrophic cardiomyopathy and hypertension) have an increased incidence of ventricular arrhythmias and sudden deaths [Von Olshausen *et al* 1983, Franciosa *et al* 1983, McLenachan *et al* 1987]. Vasodilator drug therapy and afterload-reducing agents such as angiotensin converting enzyme (ACE) inhibitors, on the other hand, has been shown to reduce mortality, including sudden deaths [CONSENSUS-1 1987, Cohn *et al* 1991]. It is also intriguing to note that while QTd is increased in these cardiac diseases [Buja *et al* 1993, Barr *et al* 1994], the reduction in mortality by drug therapies such as ACE inhibitors is paralleled by a similar reduction in QTd [Barr *et al* 1997]. It is generally thought the increased QT dispersion in these conditions occurs as a result of chronic changes in the myocardium such as left ventricular hypertrophy [Davey *et al* 1994]. Although this has been demonstrated in previous studies [Davey *et al* 1994], we believe that ours is the first to show QT dispersion may also be affected by *acute* changes in blood pressure. Our findings may therefore be relevant in a wide variety of other clinical settings where there are associated acute changes in blood pressure (eg stress, "white coat" hypertension etc).

7.4.2 Vagal Activity And QT Dispersion

In our study, atropine reduced the resting QT_{max} (and QT_{min}) but increased the Bazett-corrected QT_{cmax} (and QT_{cmin}) significantly, an effect that has also been observed in other studies [Browne *et al* 1983, Annala *et al* 1993]. However, the QT intervals were not significantly changed when linearly corrected. Changes in QT intervals are highly dependent on heart rate changes with QT interval shortening occurring as heart rate increases with atropine. Although the primary aim of our study was to look at the effects on QT dispersion, our findings with the QT interval highlight the difficulty of rate-correction in studies such as this where pharmacological interventions change heart rate significantly. As discussed above, the increase in Bazett-corrected QT_{cmax} for instance, is thought to be due to over-correction by the Bazett formula [Browne *et al* 1983, Ahnve and Vallin 1982]. This has led many investigators to question the appropriateness of heart rate correction formulae such as Bazett's which can often be misleading. This inappropriateness is further emphasized by our results which showed that atropine prolongs the Bazett corrected QT_{cmax} but not the linear corrected QT_{cmax} ; on the other hand, phenylephrine prolongs the linear corrected QT_{cmax} but not the Bazett corrected QT_{cmax} .

Because of these problems, it remains uncertain whether vagal activity does indeed modulate ventricular repolarisation; whereas some experimental data suggest that the cholinergic effects on ventricular repolarisation are minimal [Rosen *et al* 1992], others have found that atropine shortens the ventricular effective refractory period and QT_{max} interval during fixed atrial pacing [Browne *et al* 1983, Ahnve and Vallin 1982]. Our data does not clarify this particular issue but this was not the main purpose of the study.

QT dispersion, unlike the QT interval, is not heart rate dependent (as reflected by the fact that we have found no discrepancy between our results with QTd, Bazett corrected QTcd or linear corrected QTcd). In our study, QT dispersion was unaffected by atropine. Although the effects of atropine on the QT interval have been extensively investigated, studies looking at its effects on QT dispersion are limited. Our results are consistent with the observations made by Uemura *et al* [1997] in the only study that we know of that had directly examined the effects of atropine on QT dispersion in man.

The reflex vagal response to phenylephrine has also been previously investigated. Kautzner *et al* [1997] found that QT dispersion was unaffected by phenylephrine. Although this may initially appear to contradict our results, the findings are actually consistent with our study. Kautzner *et al* [1997] used boluses instead of infusions of phenylephrine in order to produce rapid vagal stimulation and to study whether vagal stimulation altered QT dispersion. Boluses of phenylephrine do not produce sustained increases in afterload and it is likely that afterload changes have to be maintained for more than a few seconds to affect QT dispersion. Hence it is not surprising that Kautzner *et al* [1997] did not find any change in QT dispersion because they were using phenylephrine boluses to study the effect of the vagus rather than using phenylephrine to study the effects of afterload as we were. The finding that reflex vagal activity did not influence QT dispersion is in fact, consistent with our observations.

7.4.3 Aldosterone And QT Dispersion

In *Chapter 3*, aldosterone blockade reduced QT dispersion. As discussed in *Chapter 1 (Section 1.5)*, there are a number of potential mechanisms in which aldosterone may cause QT dispersion. This may be the result of autonomic modulation [Bonnar *et al* 1997], electrolyte depletion [Choy *et al* 1997] or promotion of myocardial fibrosis [Brilla and Weber 1992]. Animal data even suggest that aldosterone may have direct arrhythmic effects on the heart. Aldosterone levels are higher in the heart than in plasma [Silvestre *et al* 1998]. The detection of mineralocorticoid receptors in the heart [Agarwal and Philippe 1979] as well as 11-hydroxysteroid dehydrogenase activity [Walker *et al* 1991] and the recent discovery of local aldosterone synthesis by the myocardium in animal models [Silvestre *et al* 1998], certainly suggest that aldosterone may possess paracrine or autocrine properties, with direct effects on the heart, in addition to its renal and autonomic modulating properties.

The pro-arrhythmic effects of direct administration of aldosterone infusion was demonstrated by Arora and Somani [1962] who in an experimental animal model, found to be even more striking and prolonged compared to other arrhythmogenic agents such as epinephrine. As the arrhythmogenic effect of the infusion was of rapid onset, it was concluded by the authors that electrolyte depletion was an unlikely explanation for this observation.

In this chapter the direct effects of intravenous aldosterone infusion on QT dispersion in man were examined. Despite achieving plasma levels comparable to those of CHF patients, the aldosterone infusion did not appear to have any significant effect on QT dispersion compared to placebo. This is perhaps not surprising as the

study was an acute study only and it was carried out at rest in normal healthy volunteers who are clearly very different compared to the CHF patient who is characterized by a complex and abnormal neurohormonal activated state. Furthermore it is likely that aldosterone-induced changes in the QT interval may only become observable chronically, once aldosterone has affected serum potassium. In *Study 1*, changes in QT dispersion have been shown to correlate with blood pressure changes and in *Chapter 5*, we found that aldosterone blunted the baroreflex change in heart rate. Hence a possible *direct* arrhythmogenic effect of aldosterone on the heart particular during an “activated” state (eg. during stress, exercise etc), remains to be clarified in future studies.

At present however, we can conclude that aldosterone may contribute towards QT dispersion via a number of indirect mechanisms. Aldosterone may cause both increased afterload (by increasing vascular tone and potentiating vascular smooth muscle cell hypertrophy) and preload (salt and water retention), myocardial fibrosis, electrolyte depletion and sympathetic activation: all of which may potentially increase QT dispersion. Aldosterone also impairs the parasympathetic nervous system but the parasympathetic tone does not appear to contribute towards QT dispersion.

7.4.4 Study Limitations

There are several points worth discussing. Firstly, as mentioned above, the studies in this chapter are carried out in normal volunteers who are clearly different from CHF patients who may not respond in the same manner. The results of the studies will therefore need to be replicated in CHF patients.

Secondly, although *Study 1* was designed primarily to assess the effects of afterload it must be borne in mind that any haemodynamic change in afterload will tend to affect pre-load as well. Although it is not possible to totally isolate the effects of one without changing the other in clinical studies, preload changes should be minimal in this study since phenylephrine is an alpha-adrenergic agonist which acts primarily on the arterial circulation.

Thirdly, the technical difficulty of obtaining a precise and reproducible determination of the end of the T wave measurement is well recognised. Care has been taken to use consistent criteria to define the end of the T wave and to exclude U waves. We were careful not to miss any flat U waves which may have become more obvious following the phenylephrine infusion as this may produce an artifactually short QT interval when a flat U wave became more obvious. Fortunately, this was not a major issue as the T and U waves were essentially normal and easy to measure at baseline in a cohort of normal healthy subjects. Following phenylephrine infusion, both the T and U waves become more prominent making measurements even easier. If the end of T wave in any lead were in doubt, the lead was excluded from analysis.

Finally, a direct electrophysiological effect of phenylephrine on the myocardium could not be entirely excluded. In isolated ferret Purkinje fibers, alpha-adrenergic stimulation has been shown to induce early afterdepolarizations (EAD) which may be a trigger for arrhythmia initiation [Drouin *et al* 1996]. However these effects are thought to be species specific, and have not been shown to occur in human myocardium [Jakob *et al* 1988]. Furthermore, EADs are heart rate dependent and only occur when there is prolongation of the action potential (ie. during bradycardia) [Charpentier *et al* 1993]. In our study, the effects of phenylephrine on QT dispersion

were independent of heart rate (ie. the effects were observed even when heart rate was kept constant). This means that a direct electrophysiological effect of alpha-adrenergic stimulation on QT dispersion is unlikely.

7.4.5 Conclusions

We have shown conclusively that there is a significant positive correlation between changes in afterload and changes in QT dispersion. The findings are clinically relevant and may account for part of the reason why there are such high rates of arrhythmic complications and sudden deaths in patients with conditions associated with increased afterload such as heart failure and hypertension.

On the other hand, both an acute aldosterone infusion and acute changes in parasympathetic activity, do not appear *per se* to have any significant influence on QT dispersion.

CHAPTER EIGHT

CONCLUSIONS

The therapeutic benefits of neurohormonal modulation in chronic heart failure are now well established as evident by the effects of ACE inhibitors and beta-blockers on mortality reduction in recent trials. Despite these favourable effects on mortality, CHF remains a deadly disease. It has become clear that ACE inhibitors do not completely suppress the RAAS system. Neurohormonal re-activation occurs with chronic ACE inhibition and the phenomenon of aldosterone "escape" is well described.

Until recently, the clinical significance of aldosterone in congestive heart failure has been overlooked. The results of the recent Randomised Aldactone Evaluation Study (RALES) [Pitt *et al* 1999] clearly indicate that there is further therapeutic mileage to be gained by adding an aldosterone antagonist in patients with CHF already established on ACE inhibitors. In this study, the overall risks of death, death due to progressive pump failure, and sudden death from cardiac causes were reduced by approximately 30% among spironolactone-treated patients (compared to placebo), prompting early termination of the study.

In this thesis, I have explored some of the potential adverse effects that aldosterone may have on the cardiovascular system. The results of these studies provide valuable mechanistic insights, which may account for some of the beneficial effects seen in the RALES trial.

8.1 Summary of results

In the first two studies, described in *Chapters 3 and 4*, the effects of spironolactone on cardiac autonomic tone, QT dispersion and arrhythmic activity were assessed in two different patient populations. In both studies, full diurnal

assessment of the autonomic tone including 24hr heart rate variability and noradrenaline kinetic studies were carried out. In *Chapter 3*, I investigated the effects of spironolactone in heart failure patients already established on ACE inhibitors. The cohort of patients chosen for the study was in fact, very similar to that of the RALES trial. The results of this study confirms that aldosterone, not unlike Angiotensin II, plays an important role in the regulation of the cardiac autonomic tone. The study suggest that spironolactone has important parasympathomimetic effects. Aldosterone blockade improved heart rate variability, particularly during the morning hours when aldosterone secretion is maximal. In addition aldosterone blockade had also been shown to reduce QT dispersion, a surrogate marker of sudden death.

In *Chapter 4*, I explored the effects of aldosterone blockade in a cohort of chronic stable angina patients. The study was similar in protocol to that of the CHF study described in *Chapter 3*. In addition, the impact of spironolactone therapy on the total ischaemic burden was also assessed. The results of this study were disappointing. Aldosterone blockade did not appear to have any significant effect on ischaemic events. Similarly there was no significant effect on HRV, noradrenaline kinetics and QT dispersion measures compared to placebo in stable angina patients with preserved LV function.

The subsequent chapters (*Chapters 5-7*), consisted of a series of smaller mechanistic studies in healthy normal volunteers, designed to explore some of the possible mechanisms in which aldosterone may contribute to autonomic dysfunction and increased dispersion of the QT intervals. The study described in *Chapter 5* is the first study ever to describe the detrimental effects of aldosterone on the baroreflex in man. Baroreflex sensitivity was reduced in healthy subjects when intravenous

aldosterone infusion was administered until plasma concentration levels comparable to those seen in CHF patients were achieved. It attenuated the heart rate response to a vasopressor agent (phenylephrine) but did not have any significant effect on the reflex tachycardic (or cardiac sympathetic) response to a vasodilator, sodium nitroprusside. In *Chapter 6*, a head to head comparative study of the effects of losartan, an AT1 receptor antagonist and enalapril, an ACE inhibitor on baroreceptor dysfunction was described. Pharmacologically, the former is potentially an attractive class of drug as it works at the receptor level and should therefore, have the theoretical advantage of avoiding the neurohormonal escape phenomenon seen with ACE inhibitors resulting from Ang II production by non-ACE dependent pathways. However, in this study, there did not appear to be any significant differences between the two treatments as both therapies were equally effective in improving BRS in salt depleted normotensive subjects.

In *Chapter 7*, I examined some of the mechanisms in which aldosterone may affect QT dispersion. Aldosterone may cause regional dispersion of the repolarisation phase of the action potentials in the myocardium via a number of potential mechanisms. Changes in QT dispersion appear to correlate strongly with acute changes in phenylephrine-induced cardiac afterload (or blood pressure). However, direct aldosterone infusion and vagal activity did not influence QT dispersion.

8.2 Conclusions

It is well recognised that the RAAS and the autonomic nervous system are inextricably linked in CHF. However, the interaction between the two neuroendocrine systems is not wholly understood. The studies in this thesis provide

important new insights into the role of aldosterone in cardiac autonomic regulation.

The effects of aldosterone on the kidneys (salt and water retention, potassium and magnesium excretion) and the long-term detrimental effects on hypertension and myocardial fibrosis are well established. In addition, the studies in this thesis provide strong evidence that aldosterone also has detrimental effects on the parasympathetic nervous system. Like angiotensin II, aldosterone impairs the baroreflex response in man. This is a potentially important observation as baroreflex dysfunction predisposes the myocardium to arrhythmias. Indeed, baroreflex sensitivity (BRS) has recently been shown to correlate strongly with cardiac mortality in the large prospective ATRAMI study [La Rovere *et al* 1998]. The study suggest that aldosterone exerts its effects primarily on the parasympathetic tone with little impact on the sympathetic nervous system. Aldosterone blockade in CHF provided further evidence to support the above conclusion. Studying a similar cohort of CHF patients to that of the RALES trial, I found that spironolactone improved the high frequency (HF) component of heart rate variability, a marker of vagal activity. Noradrenaline kinetics on the other hand, were unaffected.

Another interesting observation from the latter study was that QTc intervals and dispersion on the 12-lead ECG were also reduced by spironolactone therapy. Both QTcmax and QTd have been identified as sensitive predictors of sudden death [Naas *et al* 1998, Schwartz and Wolf 1978, Barr *et al* 1994]. The dispersion of ventricular action potential provide a substrate for re-entrant circuits and the development of malignant arrhythmias [Han and Moe 1964, Kuo *et al* 1983]. Mechanisms in which aldosterone may contribute towards dispersion of the QT interval are not entirely known and may be multifactorial. It is well established in

experimental models that alterations in cardiac afterload may affect the action potential duration through mechano-electrical feedback [Lab 1982]. In this thesis, I have provided evidence that changes in cardiac load would alter QT dispersion. Aldosterone may cause both increases in cardiac afterload (by increasing vascular tone and potentiating vascular smooth muscle hypertrophy) and pre-load (salt and water retention).

The effect of aldosterone on the parasympathetic nervous system is thought to be another possible mechanism, although in this thesis, I have demonstrated that vagal tone modulation itself did not contribute towards QT dispersion. Similarly, although the pro-arrhythmogenic effects of *direct* administration of aldosterone infusion has been demonstrated in experimental models [Arora and Somani 1962], an acute intravenous aldosterone infusion did not affect basal QT intervals or dispersion indices. Other potential mechanisms in which aldosterone may contribute towards QT dispersion include promotion of myocardial fibrosis and electrolyte depletion (hypokalemia, hypomagnesaemia), both of which may take time to develop. It was unfortunately beyond the scope of the thesis to explore these possibilities, which may represent ideas for future studies.

It was also intriguingly observed in the CHF study (*Chapter 3*), that heart rate was reduced and the beneficial effects of aldosterone blockade on the various arrhythmic and autonomic surrogate markers (heart rate variability, QT dispersion) were maximal during the morning hours of the day (6-10am), coinciding with the circadian pattern of cardiovascular events, such as sudden deaths and myocardial infarctions.

Hence by exerting its maximal effects during these crucial hours,

spironolactone may particularly reduce the number of such events in the dawn period of the day. Indeed the heart rate reduction during these hours raises the possibility that aldosterone blockade may have anti-ischaemic properties. Unfortunately, when tested on a cohort of stable angina patients (*Chapter 4*), no such benefit was evident. There were a number of possible reasons that may account for the discrepancy in the findings between the two studies. The two cohort of patients studied were different – unlike CHF patients, stable angina patients with preserved left ventricular function had much lower circulating aldosterone levels, a larger proportion was established on beta-blockers (which may influence HRV and reduce myocardial ischaemia), and only a minority was taking concomitant ACE inhibitors. On the other hand, the beneficial effects of spironolactone therapy in both the RALES trial and the mechanistic study in *Chapter 3* were described in CHF patients who were all fully established on ACE inhibitors.

8.3 Clinical implications and future directions

Spironolactone has been in clinical use for over 30 years, principally in the therapy of hypertension and congestive heart failure. Although simple and cheap, its use has often been neglected by physicians in the post-ACE inhibitor era, as it was thought that the latter class of drugs would provide adequate suppression of the RAAS, and hence reduce both circulating Ang II and aldosterone levels. We now know this is not the case. Combination therapy of low dose spironolactone (25-50mg/day) and an ACE inhibitor is well tolerated [Pitt et al 1999] and the results of the recent RALES trial indicate that the mortality benefits of aldosterone blockade in patients with CHF are additive to those of ACE inhibitors. This observation is an

important therapeutic advance and has lead to a resurgence of interest in the use of this old drug particularly, in the management of patients with severe CHF (NYHA grades III-IV).

The studies in this thesis provide us with some mechanistic insights as to why aldosterone blockade may be beneficial in heart failure. The detrimental effects of aldosterone are most evident during the early morning hours when there is a shift in the sympathovagal balance. Aldosterone impairs the vagally-mediated baroreflex response and reduces heart rate variability in man. It would appear that its main effect is on the parasympathetic nervous system. Although there has been some previous animal data which suggests that it may also have detrimental sympathetic effects, I was unable to confirm this.

Nonetheless, the observation that spironolactone has parasympathomimetic properties is a potentially important one. The parasympathetic nervous system plays an important role in the regulation of myocardial electrical stability. Reduced parasympathetic activity is associated with myocardial electrical instability and ventricular tachyarrhythmias, and may therefore play an important role in the pathophysiology of sudden death in CHF. Certainly reduced HRV and BRS have both been found to be good predictors of sudden deaths in CHF [Nolan *et al* 1998, La Rovere *et al* 1998]. This view is further strengthened by the fact that to date, all the other drugs that are known to reduce mortality in CHF (beta-blockers and ACE inhibitors), also regulate the autonomic tone and have been demonstrated to have favourable effects on the above surrogate markers. As the autonomic effects of spironolactone are maximal during the morning hours, it would be of interest to see if spironolactone-treated CHF patients have different diurnal pattern of deaths from

non-spironolactone treated patients. The timing of deaths were unfortunately not recorded in the RALES study.

So should we be prescribing spironolactone to all our heart failure patients?

In the RALES trial, only those at the more severe end of the disease spectrum (NYHA Class III and IV) were studied. In this thesis, the beneficial effects of aldosterone blockade on cardiac autonomic regulation were also demonstrated in a similar cohort of patients. At present there is no data that spironolactone would benefit those with asymptomatic LV dysfunction or mild heart failure (NYHA II or less). Thus, extrapolation from RALES cannot be made with confidence for non-severe heart failure. Indeed as suggested by the data from the study of stable angina patients (with normal or near-normal LV function) in this thesis, the ability of spironolactone to activate the renin-angiotensin system certainly argues against its use in the *absence* of ACE inhibition or angiotensin-receptor blockade, at least in angina patients. It remains to be seen if spironolactone would benefit patients with milder forms of heart failure (eg. asymptomatic LV dysfunction, where less circulating aldosterone levels are present). The effects of spironolactone on autonomic tone and mortality in these patients would certainly be interesting to explore in future studies.

Similarly extrapolation from the RALES data cannot be made for diastolic heart failure. Theoretically, an agent that prevents myocardial fibrosis may have particular application in this setting. However this issue was not addressed in this thesis and the RALES trial only included patients with clear systolic dysfunction (LVEF<35%). This topic too would be an interesting avenue for further research.

For the time being, the use of spironolactone is only indicated for patients

with severe systolic heart failure (NYHA Class III and IV). When and at what stage should the drug be introduced? The list of effective drug therapies in our armamentarium is growing rapidly. The benefits of ACE inhibitor therapy are well established, and this class of drugs should remain as first-line therapy for all patients with CHF. In both the RALES and heart failure studies carried out in this thesis, spironolactone was given as an *add-on* treatment in patients who had already been established on ACE inhibitors. What about the role of beta-blockers? The report from the RALES coincided with the now overwhelming evidence of benefit from beta-blockade for patients with NYHA Class II-III chronic heart failure. Should beta-blockers be introduced concurrently with spironolactone? Although only a small proportion of the RALES patients (11%) received beta-blockers, the RALES subgroup analysis did suggest that the use of both treatments may actually be synergistic in reducing mortality as the subgroup had the same proportional reduction in mortality as did those not receiving a beta-blocker. This is perhaps not too surprising as the main effect of beta-blocker therapy is on the sympathetic system whereas the main effect of spironolactone appears to be on the parasympathetic tone. However these encouraging observations for combining both treatments require confirmation in further studies. Introduction of beta-blockers requires cautious, protracted dose-titration for CHF patients. Hence, until further data becomes available, separate stepped introduction of the two drugs would seem appropriate.

Another issue worth exploring in future studies is the possible interaction between digoxin and spironolactone therapies. In RALES, it was intriguingly observed that the patients receiving both spironolactone and digoxin therapies fared better than those on spironolactone only (no digoxin). As it is well recognised that

digoxin has vagomimetic properties, it is not inconceivable that combining both therapies may be beneficial as they potentiate each other's autonomic effects. Studies evaluating the impact of the combined treatments on heart rate variability and baroreflex function, for instance, would clarify the issue.

It is worth pointing out that although the main thrust of this thesis had been the exploration of its role in autonomic regulation, aldosterone clearly has other detrimental effects on the cardiovascular system. The effects of aldosterone blockade on QT interval and dispersion indices, for instance may be related to its effect on potassium. Although this was beyond the scope of this thesis, there is certainly evidence that intravenous potassium infusion improves QT dispersion in normal subjects [Choy *et al* 1997]. It would therefore be interesting to assess if the autonomic benefits of spironolactone are also shared by other potassium sparing diuretics such as amiloride. If indeed this was so, then there may be a case for new mortality trials with other potassium sparing diuretics in heart failure in the near future.

In summary, there have been many new and exciting therapeutic advances in the management of chronic heart failure in recent years. Despite these developments, the pathophysiology of CHF remains complex and not fully understood; the work embodied in this thesis for example, highlights how inextricably linked the RAAS and the autonomic nervous system is. Neuroendocrine dysfunction contributes to haemodynamic decompensation and progression of the disease. Strategies aimed at limiting neuroendocrine disturbance in CHF are associated with an improvement in prognosis. Implementation of optimum treatment is challenging.

New therapies and innovations are currently being evaluated. These include endothelin antagonism, dual ACE and endopeptidase inhibition, and the combination of ACE inhibition and with angiotensin-receptor blockade. The mortality reduction seen with parasympathomimetic drug therapies, such as ACE inhibitors and spironolactone, suggest that enhancing the parasympathetic tone is cardioprotective and this may represent yet another therapeutic approach to the management of heart failure. Further studies should be aimed at evaluating the role of newer and more potent parasympathomimetic agents in CHF.

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APPENDIX:

Publications arising from this thesis

Can drug effects on mortality in heart failure be predicted by any surrogate measure?

Introduction

In chronic heart failure, the impact of the drug therapy on mortality is a crucial issue that can best be answered by large expensive mortality trials. The need for these large expensive trials can act as a hindrance towards novel drug development. Indeed, the daunting prospect of such an expensive trial may explain why the pharmaceutical industry has produced 20 different ACE inhibitors rather than 20 novel compounds all acting via different mechanisms. There are, however, surrogate markers which might help circumvent this problem. These non-invasive surrogate markers could help in two ways. Firstly, it may be possible for a marker to give a reasonably accurate indication of what effect the proposed new drug would have in any subsequent large mortality trial. We would then be able to select out promising drugs for these mortality studies from a large range of candidate novel therapies and avoid the current situation where financial pressures may stifle novel drug development indiscriminately. Secondly, successful markers of mortality might themselves be potential targets for future drug development in the expectation that some of these markers represent key processes leading to mortality.

However, in order for these markers to be accepted as surrogates for cardiac mortality, there must be sufficient evidence to show that drug therapy which alters mortality also causes a corresponding change in the surrogate. Not too long ago ejection fraction was considered a potential surrogate endpoint for survival in patients with heart failure. However, its positive predictive value for sudden death is low and inotropic drug therapy such as milrinone, which improved ventricular function, had the opposite, adverse effect on mortality^[1]. Hence, surrogates other than ejection fraction will need to be considered. Recent compelling evidence linking the autonomic nervous system and cardiac mortality including sudden death^[2-5], suggests to us that parameters such as heart rate variability, baroreceptor sensitivity and ventricular repolarization characteristics (QT dispersion) may well serve this purpose as potential surrogate markers. However, the predictive value of many of these autonomic markers is at present, uncertain.

The objective of this article is to assess how accurate these potential surrogate markers actually are at predicting drug effects on mortality in chronic heart failure. We have attempted to do this by reviewing the different drugs that have had an impact on mortality in heart failure and to see what their effects are on various potential surrogate markers. The markers that we have looked at include heart rate and its variability, baroreceptor sensitivity, QT dispersion, arrhythmias, late potentials and neuro-hormones.

The effect of drug therapy on various surrogate markers in chronic heart failure (see Table 1)

Drugs that have a favourable effect on mortality

ACE inhibitors

The mortality benefits of ACE inhibitors have been attributed to both neurohormonal suppression and vasodilatation^[6]. Furthermore, it has been shown that the effect of ACE inhibitors on mortality reduction was greater in those with a high baseline circulating norepinephrine level or an activated renin angiotensin system^[7]. This reduction in mortality is paralleled by changes in various markers of autonomic tone, including a decreased heart rate^[8], increased heart rate variability^[9-11], increased baroreceptor sensitivity^[12-14], decreased QT dispersion^[15] and a reduction in malignant ventricular arrhythmias^[16-18]. In summary therefore, all potential surrogates are favourably altered by ACE inhibitors, which correspond with their favourable effect on mortality^[6,8,19].

Hydralazine-isosorbide dinitrate

The vasodilator combination of hydralazine and isosorbide dinitrate has been shown to reduce mortality in the V-HeFT I study^[20]. It has no impact on arrhythmic events in patients with left ventricular dysfunction and a history of inducible ventricular tachycardia^[21]. However, unlike ACE inhibitors, the hydralazine plus isosorbide dinitrate combination is associated with a small but significant rise in heart rate and an increased plasma norepinephrine concentration during the first year of follow-up^[6]. However, the impact on autonomic markers such as heart rate

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Table 1 The effects of drug therapy on mortality and potential surrogate markers in heart failure

Drug	Mortality	Heart rate variability	Heart rate	baroreceptor sensitivity	QT dispersion	Holter arrhythmias/signal-averaged ECGs	Neuro-hormones
ACE inhibitors	G	G	G	G	G	G	G
Beta-blockers	?G	G	G	↓↑	G-n	G	G
Digoxin	O	G	O/G	G	?	O	G
Diuretics	?	?	O/G	?	?	↓↑	B
Metoprolol	B	G	O	?	?	O	G
Calcium antagonists	O	O-n	O/B	G	?	?	O
Verapamil	G	?	B	?	?	O	B
Diltiazem	B	?G	B	?	?	?	B
Amiloride	B	?	?	?	?	B	B
Class I: Flecainide	B	B	O	?	?	↓↑	?
Class III: Amiodarone	O/G	G	G	?	G	G	?

G=good effect; B=bad effect; O=neutral effect; ?=not known; ↓↑=conflicting data; n=effect documented in non-CHF patient.

variability and baroreceptor sensitivity is not known. In summary, unlike the situation with ACE inhibitors, there is a discrepancy here between their favourable effects on mortality and their unfavourable effects on heart rate and neurohormones.

Beta-blockers

In addition to heart rate reduction^[22,23], beta-blockers have consistently improved heart rate variability^[10,24,25] and plasma neurohormones (e.g. ANP, catecholamines)^[26,27]. Beta-blockers have also been reported to reduce ventricular tachyarrhythmias^[28,29]. The reports on the effects of beta-blockers on baroreceptor sensitivity are, however, conflicting^[30,32]. With regard to QT dispersion, beta-blockers have also been shown to reduce QT dispersion in the long QT syndrome^[33,34]. This has been demonstrated by Napolitano *et al.*^[33] who found that beta-blockers caused a greater reduction in those with higher baseline values. Sotalol also reduces QT dispersion in post-myocardial infarction patients^[35].

In chronic heart failure, the corresponding effects on mortality are not entirely clear, although favourable effects are likely. Metoprolol^[22] and bisoprolol^[23] have produced favourable but not statistically significant data on mortality. However, more recently, carvedilol^[36], a second generation beta-adrenergic antagonist with α_1 -blocking effects, has been shown to reduce mortality by 65% when compared to placebo in chronic heart failure. Therefore as far as beta-blockers are concerned, there is good consensus between their effects on mortality and their effects on all surrogates.

Drugs that have no overall impact on mortality

Recently the use of digoxin therapy in chronic heart failure and sinus rhythm has been controversial.

Digoxin has been shown to reduce heart rate significantly in some studies^[37,38] but not in others^[39]. Furthermore, it has also been shown to have favourable autonomic modulating properties; it restores heart rate variability^[37,38], improves baroreceptor sensitivity^[40] and reduces circulating catecholamines^[37,41,42]. However despite these promising signs, digoxin had no impact on mortality in the DIG trial^[43] or on arrhythmic events in the DIMT study^[42].

Calcium channel antagonists

The use of Ca-channel blockers in heart failure has been controversial because of their potentially negative inotropic activity and their ability to activate the neurohormonal system^[44]. However, recent evidence has shown that the second generation dihydropyridines (e.g. amlodipine or felodipine) possess more favourable neurohormonal effects^[45,47]. They appear to have no adverse effects on plasma norepinephrine levels in either normal subjects^[46] or heart failure patients^[45,47]. The long-term impact of these drugs on heart rate has not been reported from large trials. Although some dihydropyridines are known to cause an acute reflexogenic rise in heart rate, there are several small studies which show that the heart rate may subside in the long term^[48]. The effects of calcium antagonists on heart rate variability in chronic heart failure is not known, but felodipine has been shown to have no significant effect on heart rate variability in post-myocardial infarction patients^[69]. Similarly, Cook *et al.*^[49] could not show any effect with diltiazem in normal subjects. Only verapamil, which is not used in heart failure, has been shown to improve heart rate variability in post-myocardial infarction patients^[69]. There are some data suggesting that calcium antagonists improve baroreceptor

sensitivity^[50,52] in heart failure. In mortality trials, amlodipine and felodipine appear overall to have a neutral effect^[53,54].

Amiodarone

Amiodarone deserves a separate mention. Low dose amiodarone was shown to reduce total mortality (including sudden death) by 28% over a 2 year period in the GESICA trials^[55]. However, controversy has arisen, as another trial, CHF-STAT^[56] showed no improvement in survival with amiodarone despite an improvement in LV ejection fraction. The discrepancy has not been fully explained, but may be partially accounted for by differences in doses and characteristics of the patients used in the two studies. Preliminary results from the recent EMIAT and CAMIAT trials of post-myocardial infarction patients (including those with LV dysfunction) have revealed a reduction in arrhythmic events, although total mortality was not reduced. Nonetheless, amiodarone appears to have a favourable effect on potential surrogate markers; it has been shown to reduce heart rate significantly in both the GESICA^[55] and CHF-STAT^[56] studies. Furthermore it also improves increase heart rate variability^[57,58] and reduces QT dispersion^[59-61].

Drugs with adverse effects on mortality

Dopamine Receptors Agonists (Ibopamine)

Ibopamine is an active dopaminergic prodrug which works primarily as a vasodilator with some inotropic activity. It has no significant effects on heart rate^[37]. Furthermore, it does not appear to have any significant proarrhythmic effects as documented by Holter monitoring and signal-averaged ECGs^[45,62]. It has also been shown to modulate the autonomic tone favourably and to reduce circulating plasma neurohormones^[42,63]. An improvement in heart rate variability (although not statistically significant) has also been documented in a substudy of the DIMIT study^[37]. Published long-term survival data are unavailable at present but the multicentre trial PRIME II (second Prospective Randomised study of Ibopamine on Mortality and Efficacy) has apparently been terminated due to adverse effects on mortality. Ibopamine would appear to be the most worrying example of disagreement between survival data and the results with potential surrogate markers, with the latter suggesting favourable effects which were far from reproduced in the mortality trial.

Flosequin

Flosequin is a novel vasodilator with inotropic properties, no longer in use because of its adverse

effects on mortality^[64,65]. It caused an increase in heart rate^[66] and is associated with increased norepinephrine levels^[65]. However, the worsened mortality is inconsistent with its effects on heart rate variability, where it appears to increase parasympathetic and decrease sympathetic tone, respectively^[66].

Positive inotropic agents (milrinone)

Despite their beneficial effects on the haemodynamics of heart failure, phosphodiesterase inhibitors such as milrinone have been shown to have adverse effects on mortality^[1]. They are known to activate the neuro-endocrine systems, especially the renin-angiotensin system^[67] and predispose the myocardium to arrhythmias^[68]. The effects of these inotropic agents on autonomic markers such as heart rate variability are unknown.

Anti-arrhythmic therapy

The use of antiarrhythmic therapy in chronic heart failure is limited. Apart from amiodarone, most antiarrhythmic drugs (especially Class I) have no significant effects on baseline heart rate but are associated with proarrhythmic effects, increased mortality and a deterioration in heart rate variability^[57].

Summary

Clearly at present, the one perfect surrogate marker for mortality remains elusive. Chronic heart failure is a complex syndrome: as such it may perhaps be too simplistic to expect any single parameter to be universally predictive of drug effects on mortality, especially when each drug works by different mechanisms.

Nevertheless, neurohormonal antagonists, such as ACE inhibitors and beta-blockers, seem to benefit both mortality and all surrogate markers of mortality. Equally, inotropic drugs and Class I antiarrhythmics appear to worsen both mortality and many surrogates. This is encouraging. However, significant discrepancies exist, particularly for digoxin, ibopamine and hydralazine-nitrates, although it is only with the latter two that diametrically opposite effects occurred, whereby favourable surrogate effects turned into unfavourable mortality effects (or vice versa). It appears appropriate to have guarded optimism about the potential use of these surrogates to predict drug effects in chronic heart failure. Given our current understanding, none of the parameters discussed above is perfect when used alone. Perhaps a battery of surrogates would be more appropriate

rather than there being any single surrogate. The most promising surrogates are heart rate variability, QT dispersion and plasma neurohormones, the first two for sudden death and the last one for death from progressive disease.

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Aldosterone blunts the baroreflex response in man

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ABSTRACT

1. Recent animal evidence suggests that aldosterone, like angiotensin II, may possess detrimental autonomic modulating properties. Aldosterone has been shown to impair the baroreceptor response in animal models. This study is designed to test the hypothesis that aldosterone directly attenuates the baroreflex *in vivo* in man.
2. Fourteen healthy male volunteers [mean age (S.D.) 25 (9) years] received intravenous d-aldosterone ($12 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) and 5% dextrose (vehicle) in a double-blind crossover fashion, co-infused with incremental doses of intravenous phenylephrine and sodium nitroprusside. Aldosterone had no significant effect on resting blood pressure, heart rate or baroreflex response to sodium nitroprusside. However, reflex responses to phenylephrine were impaired with aldosterone ($P < 0.01$) while blood pressure responses were unaltered. Baroreflex sensitivity was significantly blunted in the aldosterone group [8.36 ± 2.19 versus $10.12 \pm 2.27 \text{ ms/mmHg}$; $P < 0.04$].
3. This study confirms previous observations from animal models that aldosterone impairs the baroreflex response. High aldosterone levels may contribute to the baroreflex dysfunction in cardiovascular diseases such as hypertension and heart failure.

INTRODUCTION

It is well established that angiotensin II (ANG II) attenuates baroreflex control of heart rate and sympathetic activity [1,2], and that angiotensin-converting enzyme (ACE) inhibitors are able to improve baroreceptor sensitivity (BRS) [3,4]. However, it is now being appreciated that aldosterone too may influence the baroreflex, irrespective of ANG II.

We recently found that spironolactone improves heart rate variability (as a measure of parasympathetic activity) in patients with chronic heart failure (CHF) [5]. Although it remains inconclusive whether this was a direct effect of

autonomic modulation by aldosterone blockade or due to the diuretic effect of spironolactone (which may potentially reduce right atrial stretch and improve heart rate variability by mechanoelectric feedback [6]), there is experimental data to suggest that aldosterone has major direct effects on the autonomic nervous system and the baroreflex. In an animal model, Wang et al. [7] showed that aldosterone infusion directly reduced baroreceptor discharge from the carotid sinus in dogs.

However, no direct information has yet been reported on the effects of aldosterone on baroreflex responses in man. As with all animal studies, it is essential to determine if these observations also occur in man, especially since

Key words: aldosterone, autonomic nervous system, baroreceptors, blood pressure, heart failure, heart rate.

Abbreviations: ACE, angiotensin-converting enzyme; ANG II, angiotensin II; BP, blood pressure; BRS, baroreflex sensitivity; CHF, chronic heart failure; HR, heart rate.

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baroreceptor dysfunction is known to play a central regulatory role in the development of cardiac arrhythmias [8–10]. Thus, we have designed this study to test the hypothesis that aldosterone attenuates the baroreflex control of heart rate *in vivo* in normal man. We have examined the effects of an acute intravenous infusion of aldosterone on the vagal and sympathetic limbs of the autonomic nervous system, by assessment of blood pressure and reflex heart rate responses to phenylephrine, a vasopressor agent, and to sodium nitroprusside, a vasodilator.

METHODS

Subjects

Fourteen normal male volunteers [mean age (S.D.) 25 (9) years] were studied. None had a history of hypertension or cardiac disease, and physical examination, routine haematological and biochemical parameters, and 12-lead ECGs were normal in all subjects. Each subject provided informed consent and the study was approved by the Tayside Committee on Medical Research.

Protocol

The subjects were studied on 2 separate days, at least 72 h apart, in a placebo-controlled, randomized, double-blind, crossover fashion. Subjects were asked to refrain from alcohol, caffeine and cigarettes for 24 h and to fast for 2 h before each study day.

Subjects rested quietly in the supine position throughout the study. Two 18G intravenous cannulae were inserted into forearm veins, one in the right arm for blood sampling and one in the left arm for infusion of either aldosterone or 5% dextrose solution (as vehicle). After 45 min of bed rest, baseline values of blood pressure (BP) and heart rate (HR) were measured non-invasively in triplicate using a semi-automatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, U.S.A.) with the cuff placed around the subject's left arm. A 12-lead ECG and venous blood (15 ml) for baseline aldosterone and angiotensin II assays were also obtained.

After this, a continuous infusion of either vehicle or d-aldosterone (Tayside Pharmaceuticals) in similar volumes was commenced in the left arm. The d-aldosterone was infused at a rate of $12 \text{ pmol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$. After 45 min of infusion, further triplicate recordings of BP, HR and continuous 12-lead ECGs were obtained along with blood samples for aldosterone and angiotensin II assays.

The haemodynamic study was then started, i.e. after 90 min of supine bed rest and 45 min after commencement of the infusion. The baroreflex response to a vasopressor agent, phenylephrine, was assessed in the first half of the study. Phenylephrine was given intra-

venously by infusion into the right forearm. It was administered in stepwise 10-min infusions ($0.2\text{--}3.6 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) by use of an infusion pump (IMED, San Diego, CA, U.S.A.). The infusion was stopped when a 35–40 mmHg rise in systolic arterial pressure had been achieved. The average systolic BP, HR and R–R interval obtained from continuous ECG recordings between 8 and 10 min after each infusion dose were recorded.

After completion of these measurements with phenylephrine, HR and BP were allowed to return to baseline values for 30 min before the second phase of the study began. Intravenous sodium nitroprusside was given in stepwise 5-min infusions ($0.2\text{--}5.2 \mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) until a maximum drop in systolic BP of 25 mmHg was achieved. HR, BP and ECG recordings were obtained after 4–5 min of each infusion dose.

Baroreflex sensitivity (BRS) assessment

The R–R intervals were plotted against the systolic BP values on a graph, and a computerized curve fit was carried out to establish a linear portion of the line of best fit. Separate linear regression lines were plotted for the responses to phenylephrine and sodium nitroprusside. Traditionally, the slope derived from the linear regression line ($\Delta\text{RR}/\Delta\text{SBP}$) obtained from the vasopressor (phenylephrine) half of the haemodynamic study has been used as an index of BRS. As in previous studies [4,11], only regression lines that had a correlation coefficient of > 0.8 were used. This method of assessment of the baroreflex using an infusion of phenylephrine has previously been shown to be reproducible [11].

Aldosterone and angiotensin II assays

Five-millilitre venous blood samples in lithium heparin tubes and 10-ml venous samples in chilled glass tubes containing a solution of 0.05 mol/l *o*-phenanthroline, 2 g/l neomycin, 0.125 mol/l EDTA (disodium salt) and 2% ethanol, were collected for measurements of aldosterone and ANG II levels respectively. The samples were centrifuged at 4 °C and the plasma was separated and stored at –20 °C (aldosterone) and –70 °C (ANG II) until assayed. Commercially available radioimmunoassay kits (Sorin Biomedica, Saluggia, Italy, and Nichols Institute Diagnostics B.F., Nieuweweg, The Netherlands) were used for the aldosterone and ANG II assays respectively.

Statistical analysis

All data were analysed using the Statgraphics software package (STSC Software Publishing Group, Rockville, MD, U.S.A.). Analysis of variance at each dose increment, using subjects and treatment as within factors, and Bonferroni multiple range tests were performed to determine the significance of the effects of aldosterone on the haemodynamic response to phenylephrine and so-

dium nitroprusside. The relationships between R-R intervals and systolic BP were studied by correlation and linear regression analyses; BRS between treatment groups was analysed using the paired Student's *t*-test. Differences were considered statistically significant if *P* < 0.05.

RESULTS

Baseline measurements (Table 1)

No significant differences in baseline measurements were seen between vehicle and aldosterone infusion days. Aldosterone infusion was painless. A significant rise in plasma aldosterone levels was noted after 45 min of aldosterone infusion compared with vehicle. There was a non-significant decrease in ANG II levels after aldosterone infusion compared with vehicle.

Haemodynamic measurements (Figures 1–3, Table 2)

No significant changes in resting BP and HR recordings were observed during aldosterone infusion compared with vehicle. Systolic BP increased and decreased in a stepwise fashion in response to the phenylephrine and sodium nitroprusside infusions respectively in both groups; no significant differences in BP responses were observed in the aldosterone group compared with vehicle (Figures 1 and 2). Similarly, no significant differences in reflex HR responses to sodium nitroprusside were observed between the two groups. The slopes of the linear regression line in response to sodium nitroprusside ($\Delta\text{RR}/\Delta\text{SBP}$) were not significantly affected by aldosterone compared with vehicle [8.62 ± 4.64 ms/mmHg (means \pm S.D.) versus 9.07 ± 2.11 ms/mmHg; *P* = 0.7] (Figure 3).

However, reflex HR responses to phenylephrine were significantly impaired in the aldosterone group compared with vehicle (*P* < 0.05). BRS was significantly depressed in the aldosterone group [8.36 ± 2.19 ms/mmHg versus 10.12 ± 2.27 ms/mmHg; *P* < 0.04].

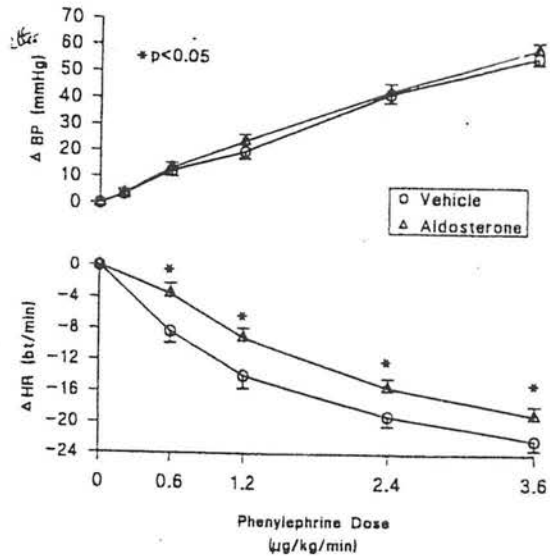


Figure 1 Change in heart rate (HR) and blood pressure (BP) responses to incremental infusions of phenylephrine. Values are means \pm S.E.M. **P* < 0.05 compared with placebo.

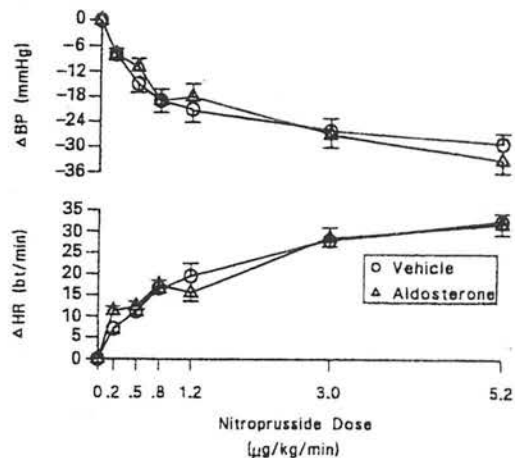


Figure 2 Change in heart rate (HR) and blood pressure (BP) responses to incremental infusions of sodium nitroprusside. Values are means \pm S.E.M.

Table 1 Baseline values

Results are expressed as means (S.D.). Statistical significance: **P* < 0.05 compared with vehicle.

	Vehicle	Aldosterone
Heart rate (beats/min)	61 (8)	60 (9)
Systolic BP (mmHg)	114 (10)	116 (5)
R-R interval (ms)	999 (156)	1021 (181)
Aldosterone levels (pg/ml)		
baseline	115 (107.8)	106.1 (81.8)
45 min after start of infusion	72.6 (62.6)	489.8 (83.3)*
ANG II levels — 45 min after start of infusion (pg/ml)	15.2 (7.6)	10.4 (3.5)

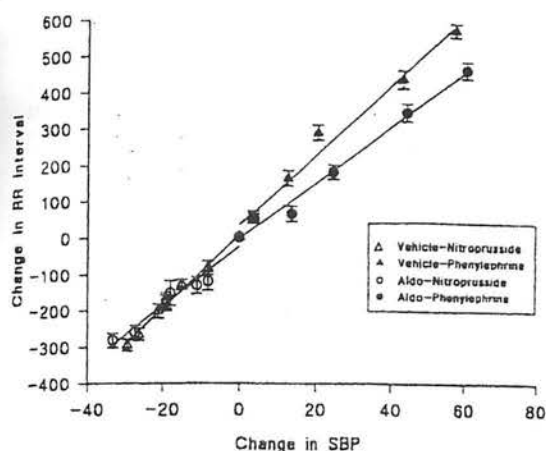


Figure 3 Relationship between changes in RR interval and systolic blood pressure (SBP)

Values are means \pm S.E.M. Separate linear regression lines for responses to phenylephrine and sodium nitroprusside for the two treatments are shown.

DISCUSSION

The present study confirms previous observations from animal models that aldosterone impairs the baroreflex response. The sensitivity of the arterial baroreceptors to a change in BP is an important determinant of the ability of the autonomic nervous system to maintain cardiovascular haemostasis.

In this study, aldosterone has been shown to impair the HR response to phenylephrine. This is in keeping with a previous observation of ours that aldosterone halved the bradycardic response to infused noradrenaline, another vasopressor agent [12]. Although acting primarily on the arterial baroreceptors, it must be borne in mind that any change in afterload by vasopressor agents will also tend to cause a corresponding change in preload and thus influence the cardiopulmonary receptors as well. On the other hand, sodium nitroprusside results in vasodilatation and generalized unloading of arterial and cardiopulmonary baroreceptors leading to increased cardiac sym-

pathetic activity [13,14]. It is interesting to note that aldosterone does not appear to have any significant effect on the reflex tachycardic (or cardiac sympathetic) response to sodium nitroprusside. These data together suggest that aldosterone exerts major effects on the parasympathetic limb of the autonomic nervous system.

With regards to its specific mechanism of action, evidence from animal studies suggests that aldosterone may have a direct action on the arterial baroreceptors. Wang et al. [7,15] not only showed that aldosterone reduces the HR response to changes in BP, but also showed conclusively that aldosterone directly reduces baroreceptor discharge from the carotid sinus of dogs. These effects were seen with both acute and chronic administration of aldosterone. On a cellular level, the mechanism is less certain. Aldosterone may elicit part of its effect on the arterial baroreceptors by stimulation of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity [7,15] as it is known to be a potent $\text{Na}^+\text{-K}^+\text{-ATPase}$ stimulant [16,17]. Depressed baroreceptor function after chronic aldosterone infusion in animal models has been shown to be partially reversed with a bolus injection of the $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor, ouabain [15]. Similarly, digoxin, another cardiac glycoside/ $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibitor, has also been shown to improve BRS in chronic hyperaldosteronaemic states such as CHF [8,18]. However, such an effect of ouabain was not seen after acute administration of aldosterone [7], suggesting that other mechanisms may be responsible for the acute effects of the hormone. Interestingly, the impaired baroreflex response due to acute administration of aldosterone could be prevented by denudation of the endothelial cells in the carotid sinus area, which has led to the suggestion that aldosterone may stimulate endothelial cells to release an unknown substance that depresses BRS activity.

Although this study was not designed to determine the specific mechanism by which aldosterone impairs the baroreflex, it has important clinical implications as it is the first study as such to extend these observations to man. This study adds to the growing body of evidence

Table 2 Change in haemodynamic parameters in response to phenylephrine infusion

Values are means and 95% confidence intervals (in brackets). Statistical significance: * $P < 0.05$, † $P < 0.01$ compared with vehicle. Abbreviations: SBP, systolic blood pressure; HR, heart rate.

	Vehicle			Aldosterone		
	Δ SBP (mmHg)	Δ HR (beats/min)	Δ RR-R interval (ms)	Δ SBP (mmHg)	Δ HR (beats/min)	Δ RR-R interval (ms)
Dose of phenylephrine ($\mu\text{g} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)						
0.2	3.5 (0.6–6.4)	3.2 (1.9–4.5)	57.9 (31.9–83.8)	3.6 (0.7–6.6)	2.7 (1.4–4.0)	50.2 (24.2–76.2)
0.6	12.5 (8.1–16.9)	8.3 (6.1–10.6)	163 (120–206)	13.5 (9.0–17.9)	3.5 (1.3–5.8)†	63.9 (20.7–107)†
1.2	20.5 (14.4–26.6)	13.9 (12.1–15.8)	288 (247–330)	24.6 (18.7–30.6)	9.0 (7.1–10.8)†	182 (141–222)†
2.4	43.1 (36.3–49.8)	19.0 (17.5–20.5)	435 (385–485)	44.1 (37.4–50.9)	15.3 (13.8–16.8)†	343 (293–393)*
3.6	57.2 (51.7–62.7)	22.1 (20.1–24.2)	571 (533–609)	60.4 (53.9–67.0)	18.8 (16.4–21.2)*	462 (416–507)†

that aldosterone, like ANG II, has autonomic modulating properties. Aldosterone has been shown to block noradrenaline uptake in the heart *in vivo* in an animal study [19]. In accordance with this, we recently found that spironolactone, an aldosterone antagonist, increased myocardial noradrenaline uptake [19] and improved HR variability [5] in patients with CHF. Although in our study, aldosterone does not appear to have any significant effect on the reflex sympathetic response to sodium nitroprusside in healthy volunteers, this finding is not necessarily a contradiction to our previous findings since infusing nitroprusside into normal man clearly does not exactly reproduce the activated sympathetic system of patients with CHF, even though nitroprusside is a traditional way of assessing sympatho-activation in man. One important difference between the two is that filling pressures are high in CHF but are likely to be subnormal after nitroprusside unloading in normal man.

There are, however, some limitations to our study worth discussing. First, the study was performed in healthy subjects and not in patient groups. Patients with CHF, for example, have a markedly abnormal and complex haemodynamic and neurohormonal state. Although it would be of interest to assess the effects in these patient groups, they are not ideal for the purpose of this study which was to assess the effects of aldosterone on baroreceptor function *in vivo* in man. In our study of healthy subjects we were able to isolate the effects of aldosterone on the baroreflex (and thus confirm the data from animal models) while avoiding the many possible confounding factors present in patients with CHF who are characterized by the presence of other circulating neurohormones, impaired vascular tone and endothelial dysfunction.

Secondly, salt intake was not controlled for in this study. Alterations in body salt status may potentially affect the renin-angiotensin-aldosterone axis. However, volunteers were advised to remain on their usual diet throughout the study and, as reflected by the baseline measurements, there were no significant differences in plasma neurohormones between the two study days.

Finally, infusions rather than boluses of pressor and vasodepressor stimuli were used in this study, allowing for baroreceptor 'resetting' to occur and hence reducing or dampening any change in baroreflex response. This may mean that small changes in baroreflex response to sodium nitroprusside, for example, may have gone undetected. On the other hand, one might expect, if anything, the blunting effect of aldosterone on baroreflex response to phenylephrine to be amplified if the bolus method had been used instead. BRS measurements by infusion method may not be equivalent to the bolus method, but the infusion method has been shown to be reproducible [11].

Infusions were used in this study because it allowed us to monitor HR and BP changes at steady state non-

invasively at each incremental dose which has the advantage that our readings were taken in triplicate, which should minimize random measurement error. In addition, the bolus method requires either invasive intra-arterial measurements (more risk and discomfort to the subject using radial artery devices) or non-invasive beat-to-beat analysis using the Finapres, which is not possible at our institute as the Finapres devices are no longer available in the U.K. Furthermore, we were more interested in changes or differences in baroreceptor sensitivities between two treatments rather than in using absolute levels of BRS to compare one population with another.

In summary, this study has established that in man *in vivo*, aldosterone has a detrimental effect on the parasympathetic component of the baroreflex response. The effects of aldosterone on the autonomic nervous system have important clinical implications. In conditions such as CHF and hypertension, it is well documented that the suppressive effect of long-term ACE inhibitors on aldosterone is weak, variable and unsustained, whether or not ANG II itself remains suppressed [20-22]. It is noteworthy that in our study, the plasma concentration of aldosterone [mean (S.D.) 489.8 (83.3) pg/ml] achieved on the treatment days was similar to those observed in patients with CHF [20].

Baroreflex dysfunction is thought to be a key process contributing to the development of ventricular arrhythmias and mortality in patients with CHF and ischaemic heart disease [8,9,23]. Thus, it follows that if residual aldosterone is partly responsible for the blunting of the baroreflex in these patients, there may well be a therapeutic benefit in considering anti-aldosterone therapy in addition to ACE inhibitors. It must be borne in mind, however, that only the effects of acute aldosterone administration have been examined in this study, and whether these observations also extend to conditions characterized by chronic elevated aldosterone levels remains to be determined. The potential mortality benefits of giving spironolactone in addition to ACE inhibitors are currently being evaluated in the multi-centre RALES study [24]. If the RALES study turns out to be positive, then this study will have highlighted an important possible mechanism for such a beneficial effect on mortality.

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Endogenous angiotensin II and baroreceptor dysfunction: a comparative study of losartan and enalapril in man

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Aims To assess the role of direct AT₁ receptor antagonism in baroreceptor modulation in man, and to perform a direct comparison of Ang II blockade at the receptor level with that of ACE inhibition.

Methods Ten healthy male volunteers [mean age (s.d.) 23 (6.9)] pretreated with frusemide therapy (40 mg day⁻¹ for 3 days prior to each visit) were studied on 3 separate days, 10 days apart, in a placebo-controlled, randomized, double-blind, cross-over fashion. On each study day, subjects were randomly given either a single-dose of enalapril 20 mg, losartan 50 mg or placebo. Baroreceptor function was assessed by measuring changes in blood pressure (BP), pulse interval (RR Int) and heart rate (HR) in response to incremental doses of intravenous phenylephrine infusions (0.2–3.6 µg kg⁻¹ min⁻¹).

Results In response to phenylephrine, no significant differences in BP responses were observed with any of the study medications but reflex heart rate responses were significantly increased with both enalapril and losartan compared with placebo ($P < 0.05$). The (RR/ΔsBP) ratio, taken as a measure of baroreceptor sensitivity (BRS) was significantly increased with enalapril [12.2 ± 4.6 ms mmHg⁻¹ (mean ± s.d.)] and losartan [11.9 ± 3.6 ms mmHg⁻¹] compared with placebo [9.2 ± 4.5 ms mmHg⁻¹]; i.e. enalapril and losartan increased the (RR/ΔsBP) ratio by 3.0 ms mmHg⁻¹ (95%CI 0.5, 5.6; $P < 0.05$) and 2.8 ms mmHg⁻¹ (95%CI 0.6, 5.0; $P < 0.038$), respectively. There were however, no significant differences between losartan and enalapril [mean difference 0.25 (95%CI -1.6, 2.1)].

Conclusions The present study confirms observations from animal models that blocking endogenous angiotensin II in man improves baroreceptor function. Both strategies, AT₁ receptor antagonism and ACE inhibition appear to be equally effective in restoring baroreceptor function in salt-depleted normotensive subjects.

Keywords: angiotensin II, baroreceptor, enalapril, losartan

Introduction

Baroreflex dysfunction is thought to be a key process leading to ventricular dysrhythmias and sudden deaths in patients with CHF and in postmyocardial infarction patients [1–3]. A major component of baroreflex dysfunction is vagal activity. In animal studies [4, 5], vagal stimulation dramatically improves survival and reduces dysrhythmias after coronary artery ligation.

It is well established that angiotensin II (Ang II) attenuates baroreflex control of heart rate and increases sympathetic activity [6, 7]. Similarly, ACE inhibitors have been shown to improve both heart rate variability and baroreceptor sensitivity (BRS) in man [8–10]. Indeed, the effect of ACE inhibitors on BRS may well be instrumental in their ability to improve mortality in heart failure.

Compared with ACE inhibitors, Ang II type 1 receptor (AT₁) antagonists offer a more selective and complete blockade of Ang II. The question naturally arises whether

AT₁ receptor antagonists will also have favourable effects on BRS. Pharmacologically, they may even be more effective at boosting BRS because Ang II levels are not completely suppressed by ACE inhibitors [11]. However, although direct AT₁ receptor antagonists have been shown to improve baroreceptor function in animal models [12, 13], the only study to examine this question in man found no effect of losartan on baroreflex sensitivity [14]. This whole question has been given added impetus by the recent Evaluation with Losartan in the Elderly (ELITE) trial results. Although designed primarily to assess safety and efficacy of the treatments and not mortality, it was intriguingly observed in the multicentre ELITE trial [15] that those randomized to losartan had a 46% reduction in all-cause mortality in comparison with captopril-treated patients, which was primarily due to a decrease in sudden cardiac deaths.

The purpose of the present study was two fold. Firstly, the main aim was to assess if AT₁ receptor antagonism really does improve baroreceptor function in man, as had been previously demonstrated in animal models although it did not appear to in the only human study of the matter [14]. The secondary aim was to perform a head to head

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comparison of Ang II blockade at the receptor level with that of ACE inhibition to see if there were any major differences between them which might be relevant to the ELITE results. In this study, a comparison of a single oral dose of losartan potassium 50 mg was made with a single dose of an ACE inhibitor, enalapril maleate 20 mg. In particular, we performed this study in normal man in whom the endogenous renin angiotensin system (RAAS) had been activated as it would enable us to assess the activated RAAS of heart failure in isolation while avoiding the confounding influences of increased age, comorbidity and polypharmacy which would be present in CHF.

Methods

Subjects

Ten normal male volunteers [mean age (s.d.) 23 (6.9)] were studied. None had a history of hypertension or cardiac disease. Physical examination, routine haematological and biochemical parameters, and 12 lead electrocardiograms (ECG) were normal in all subjects. Each provided informed consent in writing, and the study was approved by the Tayside Ethics Committee on Medical Research.

Protocol

Subjects were studied on 3 separate days, 10 days apart, in a placebo-controlled, randomized, double-blind, cross-over fashion. Three days prior to each study visit, subjects were pretreated with oral frusemide 40 mg day⁻¹ to activate their endogenous renin-angiotensin system. The subjects were instructed to take the daily frusemide dose at 18.00 h and they were asked to maintain their usual diet for the duration of the study and to adhere to the same pattern of meals in the 48 h preceding each visit day. Subjects were also required to refrain from alcohol, caffeine and cigarettes for 24 h and to fast for 2 h before each study day.

On the study day (i.e. the day after they had completed each course of frusemide tablets), the subjects attended our department at 08.00 h. Each subject was given randomly, a different tablet on each visit day which comprised of either a placebo tablet, enalapril 20 mg or losartan 50 mg.

Subjects rested quietly in the supine position throughout the study period. An 18G intravenous cannula was inserted into a right forearm vein for drug infusions and blood sampling. After 45 min of bedrest, baseline values of blood pressure (BP) and heart rate (HR) were measured noninvasively in triplicate using a semiautomatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, FL, USA) with the cuff being placed around the subjects left arm. A 12 lead electrocardiogram and venous blood (15 ml) for baseline aldosterone and Ang II assays were also obtained.

The haemodynamic and baroreceptor assessments were carried out after 6 h following ingestion of the oral medication as the haemodynamic effects of both a single dose of oral losartan and enalapril are known to peak after 6 h [16, 17]. Further triplicate recordings of resting blood pressure, heart rate and continuous 12-lead ECGs were obtained before the reflex baroreceptor response to a

vasopressor agent, phenylephrine (PE), was assessed. Intravenous PE was administered in stepwise 10 min infusions (0.2–3.6 µg kg⁻¹ min⁻¹) by use of an infusion pump (IMED, San Diego, CA). The infusion was stopped when a 35–40 mmHg rise in systolic arterial pressure had been achieved. The average systolic BP, HR and R-R interval obtained from continuous ECG recordings between 8 and 10 min after each infusion dose were recorded. After completion of these measurements with PE, HR and BP were allowed to return to baseline values.

Baroreflex sensitivity (BRS) assessment

The R-R intervals were plotted against the systolic blood pressure in a graph, and a computerised curve fit was then carried out to establish a linear portion of the line of best fit. As in previous studies [8, 9, 18], only regression lines that had a correlation coefficient of >0.8 were used; the slope of the linear portion of this relationship ((RR/ΔsBP) was taken as an index of baroreflex sensitivity (BRS). The method of assessment of the baroreflex using an infusion of PE has been previously shown to be reproducible [18].

Aldosterone and angiotensin II assays

Venous blood samples (5 ml) in lithium heparin tubes and 10 ml venous samples in chilled glass tubes containing a solution of 0.05 mol l⁻¹ o-phenantroline, 2 g l⁻¹ neomycin, 0.125 mol l⁻¹ EDTA (disodium salt) and 2% ethanol, were collected for measurements of aldosterone and Ang II levels, respectively. The samples were centrifuged at 4°C and the plasma was separated and stored at -20°C (aldosterone) and -70°C (Ang II) until assayed. Commercially available radioimmunoassay kits (Sorin Biomedica, Saluggia, Italy, and Nichols Institute Diagnostics B.F., Nieuweweg, The Netherlands) were used for the aldosterone and Ang II assays, respectively.

Statistical analysis

All data were analysed using the Statgraphics software package (STSC Software Publishing Group, Rockville, MD, USA). Multiple analysis of variance, using subjects and treatment as within factors, and Bonferroni multiple range tests were performed to determine the significance of the effects of losartan and enalapril on the haemodynamic response to phenylephrine. The relationships between R-R intervals and systolic BP were studied by correlation and linear regression analyses; BRS between the placebo and treatment groups were analysed using the paired Student's *t*-test. Differences were considered statistically significant if *P* < 0.05.

Results

Baseline measurements

Resting haemodynamic and biochemical measurements were similar at all study visits prior to administration of the study medications (Table 1). As expected, basal plasma Ang II and aldosterone levels were elevated as a result of frusemide-

Table 1 Baseline values. Results are expressed as means \pm s.d. Statistical significance: * $P < 0.004$ compared with placebo.

		Placebo	Enalapril	Losartan
Heart rate (beats min ⁻¹)	Pre-dose	67.6 (4.6)	66.6 (9.1)	69.1 (9.4)
	6 h post-dose	68.0 (7.4)	67.1 (8.4)	68.8 (7.1)
Systolic BP (mmHg)	Pre-dose	117.6 (9.9)	117.1 (7.6)	118.5 (7.1)
	6 h post-dose	119.3 (8.5)	107.5 (9.8)*	110.1 (7.4)*
R-R interval (ms)	Pre-dose	892 (62)	916.5 (122)	883 (115)
	6 h post-dose	892 (96)	907 (108)	881 (89)
Plasma Ang II (pg ml ⁻¹)	Pre-dose	42.6 (34.4)	40.7 (19.9)	44.9 (29.0)
Plasma aldosterone (pg ml ⁻¹)	Pre-dose	194 (189)	113 (63)	141 (79)

induced salt depletion. At 6 h following ingestion of study medication, resting blood pressure was significantly reduced with both losartan and enalapril by 8.4 mmHg (95% CI 4.2,

12.6; $P = 0.0038$) and 9.6 mmHg (95% CI 4.6, 14.6; $P = 0.004$), respectively, compared with placebo. However, there were no significant differences with resting heart rate either at the start or at 6 h after medication.

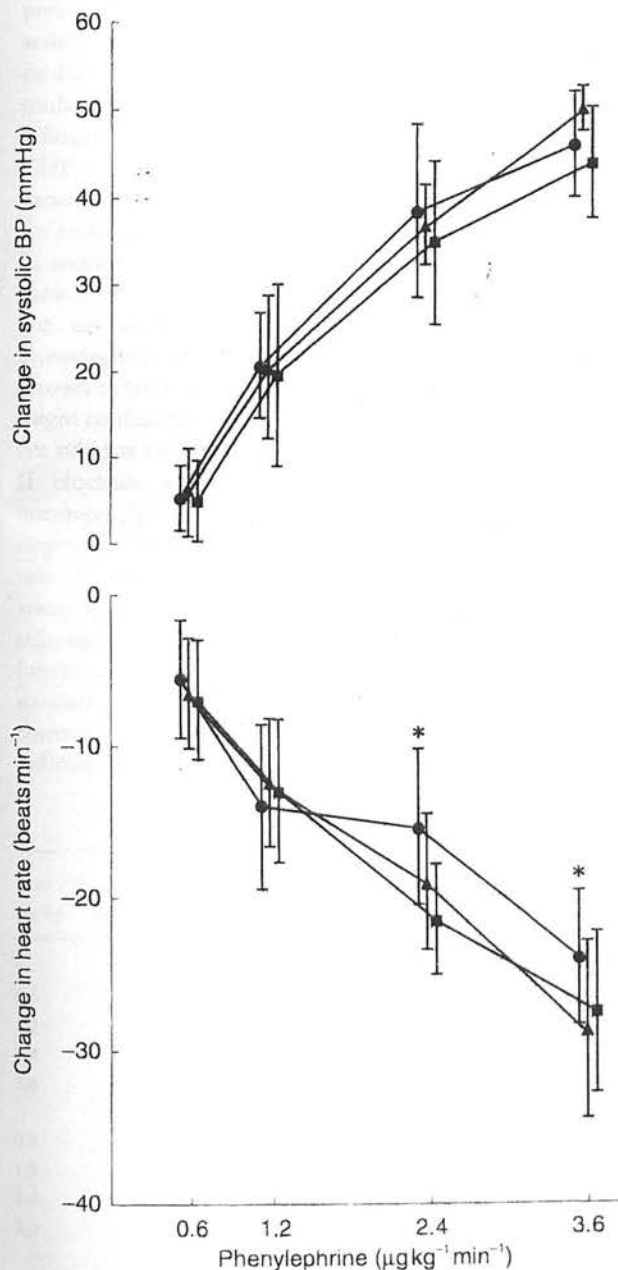


Figure 1 Change in heart rate (Δ HR) and blood pressure (Δ SBP) responses to incremental infusions of phenylephrine. Values are mean \pm s.d. \bullet placebo, \blacktriangle enalapril, \blacksquare losartan * $P < 0.05$ compared with placebo.

Baroreceptor assessment (Figures 1, 2, Table 2)

Systolic blood pressure and reflex heart rate increased and decreased, respectively, in a stepwise fashion in response to the phenylephrine infusion on all 3 study days. Whereas no significant differences in BP responses were observed with any of the study medications, reflex heart rate responses to phenylephrine were significantly increased with both enalapril and losartan compared to placebo ($P < 0.05$). The (RR/ Δ SBP ratio, taken as a measure of BRS was significantly increased with enalapril [12.2 ± 4.6 ms mmHg⁻¹ (mean \pm s.d.)] and losartan [11.9 ± 3.6 ms mmHg⁻¹] compared with placebo [9.2 ± 4.5 ms mmHg⁻¹]; i.e. enalapril and losartan increased the (RR/ Δ SBP ratio by 3.0 ms mmHg⁻¹ (95%CI 0.5, 5.6; $P < 0.05$) and 2.8 ms mmHg⁻¹ (95%CI 0.6, 5.0; $P < 0.038$), respectively. There were however, no significant differences between losartan and enalapril [mean difference 0.25 (95%CI -1.6, 2.1)]. The individual BRS indices are displayed in Figure 2.

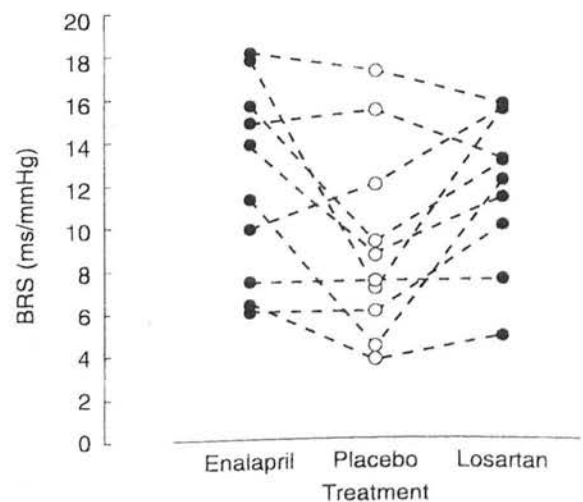


Figure 2 Individual baroreflex sensitivity (BRS) data. The individual BRS indices (slope of the linear regression line Δ RR/ Δ SBP) in response to each of the three treatments are displayed.

Discussion

In this study, the haemodynamic effects of a single dose of oral losartan potassium and a single dose of oral enalapril maleate were examined in salt-depleted normotensive subjects pretreated with diuretics. Assessments were made 6 h after oral administration of the respective medications i.e. at the time when the haemodynamic effects of the drugs are maximal [16, 17]. The hypotensive effect of a single dose of 50 mg losartan was comparable with that of 20 mg enalapril (systolic BP reduced by 8.4 mmHg [95% CI 4.2, 12.6] and 9.6 mmHg [95% CI 4.6, 14.6], respectively). In accordance with data from other studies [16, 17, 19, 20] resting blood pressure was significantly reduced by both drugs but resting heart rate was unaffected.

The absence of reflex tachycardia accompanying blood pressure reduction has been attributed to the parasympathetic activity of these drugs. The influence of Ang II on the cardiac vagal activity is well established in both animal studies [21, 22] and human studies involving steady state infusions of Ang II [7]. Although in disease states such as CHF, ACE inhibitors have been shown to enhance baroreceptor function [8, 9], the evidence for such a role for endogenous Ang II in healthy man has been conflicting. In sodium replete hypertensive subjects, captopril has been shown to cause displacement of the baroreceptor set-point but no modification of the BRS during activation by phenylephrine [23, 24]. However, hypertensive patients are known to have a blunted baroreflex function [24, 25] which might confuse the picture. Hence, only studies in normotensive subjects will allow a true assessment of the effect of Ang II blockade *per se* on baroreceptor function. Amongst normotensive subjects, Ibsen *et al.* [20] found that enalapril improved BRS function whereas Giulicelli *et al.* [26] did not. In the only human study involving AT1 receptor antagonists, losartan had no effect on baroreflex sensitivity, although this was measured by the gain of the transfer function relating BP to pulse interval rather than by more standard techniques [14]. One of the possible reasons for the contradictory data in the literature may be related to the differences in the salt status and degree of endogenous Ang

II activation in the different study populations. The latter two studies above [14, 26] were carried out in normotensives with normal salt status whereas in the study by Ibsen *et al.* [20], the subjects were mildly sodium depleted. This observation may be important suggesting that the influence of endogenous Ang II on the autonomic and vascular tone may only become prominent during an activated RAAS state e.g. during salt depletion. It has been shown in a recent animal model that Ang II blockade improved the baroreflex response to a greater extent in rats fed with a sodium deficient diet compared with those on a high sodium diet [27]. The effects of raised endogenous Ang II is clinically relevant in a cardiovascular diseases such as CHF and hypertension where the RAAS is activated and may even be further exacerbated by diuretic therapy. Recent data even suggest that the RAAS is activated in hypercholesterolaemic patients [28] which may make our findings relevant to many disease groups.

The effects of raised endogenous Ang II on reflex baroreceptor function were assessed in this study. The subjects in the study were pretreated with frusemide to activate the RAAS. The reflex bradycardic response to phenylephrine was significantly increased following treatment with enalapril and losartan. In addition, the slopes of the (RR/ Δ sBP linear regression line were also significantly increased. These observations support our hypothesis that raised endogenous Ang II levels do contribute to baroreceptor dysfunction and that Ang II blockade, either by ACE inhibition or direct antagonism at the receptor level, would reverse it. These findings are also in agreement with observations from animal models where both losartan [27, 29] and enalapril [29] have been shown to enhance baroreceptor sensitivity.

It is also interesting to note in this study that the effects of losartan were comparable with those of enalapril. Although minor differences between the two clearly could not be excluded, we were able to exclude large or significant differences. Direct Ang II receptor antagonists such as losartan lack the bradykinin potentiation seen with ACE inhibition. Although there are beneficial effects associated with bradykinins, the lack of bradykinin-related adverse

Dose PE ($\mu\text{g kg}^{-1} \text{ min}^{-1}$)	Placebo	Enalapril	Losartan
		<i>AsBP (mmHg)</i>	
0.6	5.3 \pm 3.8	5.9 \pm 5.1	5.0 \pm 4.6
1.2	20.6 \pm 6.1	20.4 \pm 8.3	19.5 \pm 10.4
2.4	38.1 \pm 9.8	36.6 \pm 4.5	34.6 \pm 9.3
3.6	45.6 \pm 6.0	49.6 \pm 2.6	43.6 \pm 6.4
		<i>ΔHR (beats min⁻¹)</i>	
0.6	5.6 \pm 3.9	6.6 \pm 3.6	7.0 \pm 3.9
1.2	14.0 \pm 5.4	12.5 \pm 4.2	13.0 \pm 4.7
2.4	15.4 \pm 5.1	19.0 \pm 4.4*	21.4 \pm 3.6*
3.6	23.9 \pm 4.4	28.6 \pm 5.8†	27.5 \pm 5.3*
		<i>IR-R interval (ms)</i>	
0.6	86 \pm 64.5	101.5 \pm 68	110 \pm 79
1.2	250 \pm 127	222 \pm 113	224 \pm 127
2.4	280 \pm 125	376 \pm 142*	421 \pm 149*
3.6	517 \pm 204	738 \pm 309*	644 \pm 269

Table 2 Changes in haemodynamic parameters in response to phenylephrine infusion. Values are mean \pm s.d. Statistical significance: * $P < 0.01$; † $P < 0.05$ compared with placebo.

effects makes AT1 receptor antagonists an attractive alternative to ACE inhibitors. The recent ELITE trial [15] suggest that losartan may be even better than ACE inhibitors in terms of reducing mortality and in particular sudden deaths in heart failure. Although baroreflex dysfunction is thought to be a key process leading to ventricular dysrhythmias and sudden deaths, our study did not show any large differences in baroreceptor modulation between the two strategies. There may be several explanations for this. Firstly, the mechanisms for sudden death are multifactorial. Secondly, the number of deaths in the ELITE trial was small as it was not designed primarily as a mortality trial and the results may therefore occur by chance. Thirdly, there are inevitably, some limitations to our study.

Infusions rather than boluses of pressor stimuli were used in this study, allowing for baroreceptor 'resetting' to occur and hence reducing or dampening any change in baroreceptor response. This may mean that potentially small differences in baroreceptor response between the two treatments for instance, may have gone undetected. BRS measurements by infusion method may not be equivalent to the bolus method, but the infusion method has been shown to be reproducible [18].

Infusions were used in this study firstly, as it allowed us to monitor HR and BP changes at steady state noninvasively at each incremental dose, and hence avoiding the need for invasive beat-to-beat intra-arterial measurements as required by the bolus method. Secondly, noninvasive beat-to-beat analysis using the FINAPRES is now difficult in the United Kingdom as the FINAPRES machines are no longer available. Therefore, although our measurements may not exactly match the beat-to-beat method, our technique has the advantage that our readings were taken at steady state and more importantly still each of our readings were taken in triplicate, which should minimize random measurement error. Furthermore we were more interested in *changes or differences* in baroreceptor sensitivities between two treatments rather than in using absolute levels of BRS to compare one population with another.

Despite these limitations, our data clearly indicate that blocking endogenous Ang II in man improves baroreceptor function. Both strategies, AT1 receptor antagonism and ACE inhibition appear to be equally effective in restoring baroreceptor function in salt-depleted normotensive subjects. This may be clinically relevant in conditions such as CHF; the long-term effects of these treatments are currently being evaluated in the ongoing mortality trial, ELITE II.

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Effect of Phenylephrine With and Without Atropine on QT Dispersion in Healthy Normotensive Men

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The present study examined if changes in cardiac afterload would affect QT interval dispersion. QT dispersion (QTd) on the 12-lead electrocardiogram is believed to be a noninvasive measure of electrical inhomogeneity in the heart and has recently been identified as a sensitive predictor of sudden cardiac death. In experimental models, an increase in cardiac afterload has been shown to alter action potential durations through mechanoelectrical feedback. This may result in an altered dispersion of action potential repolarization in the ventricle. Until now, there has been little evidence for this occurring in man *in vivo*. In the present study, the effects of afterload on QTd were examined in 10 healthy male volunteers (mean age [SD] 25 years [4.5]) who received an intravenous infusion of phenylephrine (0.2 to 3.6 $\mu\text{g/kg/min}$) given in incremental doses, and placebo in a

blinded, crossover fashion. Because phenylephrine is known to alter heart rate (HR) significantly (via a reflex vagal response), the study was performed with and without atropine. We found a significant positive correlation between acute changes in blood pressure (BP) and changes in all QTd indexes ($\Delta\text{QTd}/\Delta$ systolic BP and $\Delta\text{QT}_{\text{c,d}}/\Delta$ systolic BP r values 0.67 and 0.60, respectively; $p < 0.001$). This relation was independent of HR changes or reflex vagal activity. Atropine had no significant effect on QTd. These observations have important clinical implications and may partly account for why sudden deaths and arrhythmic complications occur so frequently in conditions associated with increased afterload, such as hypertension and heart failure. ©2000 by Excerpta Medica, Inc.

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QT dispersion (QTd), the difference between the longest and shortest QT interval measured on the 12-lead electrocardiogram, has recently been identified as a simple and easily measured predictor of sudden cardiac death.¹⁻³ QTd is believed to be a measure of electrical inhomogeneity in the heart that decreases an individual's threshold for ventricular arrhythmias.⁴ Unraveling what factors actually contribute to electrical inhomogeneity is essential if we are to develop specific therapies to overcome it, and thus reduce sudden death. One factor that may be important is cardiac afterload. In experimental models, an acute increase in cardiac afterload has been shown to be arrhythmogenic.^{5,6} An increase in cardiac loading with subsequent stretching of the ventricular myocardium causes shortening of the action potential duration,^{7,8} and this may result in an altered dispersion of action potential repolarization in the ventricle.⁹ Until now, there has been little evidence for this occurring in man *in vivo*. We examined whether changes in cardiac afterload would alter QTd by examining the effects of an acute phenylephrine-induced increase in afterload on QTd in 10 healthy men. Because phenylephrine is known to alter heart rate (HR) signifi-

cantly (via a reflex vagal response), the study was performed with and without atropine. We also examined whether atropine per se would alter the QTmax interval and QTd.

METHODS

Subjects: Ten normal male volunteers (mean age [SD] 25 years [4.5]) were studied. One patient was withdrawn from the study because he could not tolerate the phenylephrine infusion (his systolic blood pressure (BP) increased by >50 mm Hg at the lowest dose administered). No patient had a history of hypertension or cardiac disease, and physical examination, routine hematologic and biochemical parameters, and 12-lead electrocardiograms were normal in all subjects. Each patient provided informed consent, and the study was approved by the Tayside Ethical Committee on Medical Research.

Protocol: Subjects were studied on 2 separate days, at least 72 hours apart, in a placebo-controlled, randomized, single-blinded, crossover fashion. Subjects were requested to refrain from alcohol, caffeine, and cigarettes for 24 hours and to fast for 2 hours before each study day. Subjects rested quietly in the supine position throughout the study. A single 18-gauge intravenous cannula was inserted into a right forearm vein of each subject. After 30 minutes of bedrest, baseline values of BP and HR were measured noninvasively in triplicate using a semiautomatic sphygmomanometer (Dinamap Vital Signs Monitor 1846; Critikon, Tampa, Florida) with the cuff being placed around the subject's left arm. Baseline 12-lead electrocardiograms were also obtained. Following this, a

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TABLE I Baseline Hemodynamic and Electrocardiographic Measurements With Placebo Infusion

	Preinfusion (Start)	Post-Atropine*	p Value
Systolic BP (mm Hg)	114 ± 10	116 ± 11	NS
Diastolic BP (mm Hg)	64 ± 12	66 ± 9	NS
HR (beats/min)	66 ± 12	109 ± 10	<0.001
QT _{max} (ms)	401.5 ± 33	347 ± 19	<0.001
QT _{max} (linear correction)	416 ± 19	421 ± 17	NS
QT _{max} (Bazett's correction)	412 ± 14	448 ± 15	<0.001
QT _{min} (ms)	357 ± 20	301 ± 24	<0.001
QT _{min} (linear correction)	371 ± 15	375 ± 18	NS
QT _{min} (Bazett's correction)	365 ± 22	395 ± 21	<0.01
QTd (ms)	45 ± 15	46 ± 6	NS
QT _d (linear correction)	45 ± 15	46 ± 6	NS
QT _d (Bazett's correction)	47 ± 15	54 ± 10	NS

*Denotes values at rest following atropine before recommencement of placebo infusion (see text).

Expressed as mean ± SD. Statistical significance ($p < 0.05$) refers to comparison with preinfusion baseline values. Baseline values at rest during phenylephrine infusion are similar (see Table II).

continuous infusion of either phenylephrine (Knoll Pharma, Nottingham United Kingdom) or placebo (5% dextrose) in similar volumes, was administered intravenously. Up to 6 incremental stepwise 10-minute infusions (0.2 to 3.6 $\mu\text{g/kg/min}$) were administered using an infusion pump (IMED, San Diego, California). The infusion was halted when a 35 to 40 mm Hg increase in systolic BP had been achieved with the active treatment. Infusions of the placebo were performed in similar volumes and incremental steps as the active treatment. BP, HR, and electrocardiographic recordings were obtained in triplicate between 8 and 10 minutes after each infusion dose. After completion of these measurements, the infusion was discontinued and BP and HR at rest were allowed to return to baseline values for 30 minutes.

Atropine 0.03 mg/kg was then administered by slow IV injection over 1 minute. Once HR and BP had restabilized following full vagal blockade, a repeat baseline 12-lead electrocardiogram was obtained and the infusion of phenylephrine (or placebo) was restarted and the study repeated again as described above. Forty-five minutes after the injection of atropine, a further 0.6 mg of atropine was given intravenously to ensure continued vagal blockade. At the end of the study BP and HR at rest were allowed to return to normal.

QT interval and dispersion analysis: Electrocardiograms (with simultaneous 12-lead acquisition) were recorded with a Hewlett-Packard 4700A electrocardiographic machine (Palo Alto, California). All measurements were analyzed blindly by a single observer (KMY) on a Calcomp digitizing tablet (Twyford, Berkshire, United Kingdom) using customized software, as described elsewhere.^{2,3} The QT interval was taken from the onset of the QRS to the end of the wave (i.e., return to the T/P baseline). If U waves were present, the QT interval was measured to the nadir of the curve between the T and U waves. Three consecutive cycles were usually measured for each lead. QT

TABLE II Effects of Phenylephrine (\pm Atropine) on Heart Rate and QT Intervals

	Baseline (no atropine)	Phe	p Value
HR	58 ± 11	42 ± 4	0.0004
QT _{max}	418 ± 27	460 ± 26	0.0003
QT _{max} (linear correction)	415 ± 20	429 ± 25	0.017
QT _{max} (Bazett's correction)	410 ± 22	400 ± 28	NS
QT _{min}	373 ± 25	396 ± 24	0.001
QT _{min} (linear correction)	369 ± 20	364 ± 20	NS
QT _{min} (Bazett's correction)	364 ± 24	332 ± 25	0.001

	Baseline (with atropine)	Phe-ATR	
HR	104 ± 13	106 ± 13	NS
QT _{max}	345 ± 27	348 ± 25	NS
QT _{max} (linear correction)	422 ± 18	429 ± 13	NS
QT _{max} (Bazett's correction)	455 ± 24	465 ± 14	NS
QT _{min}	299 ± 24	285 ± 17	0.038
QT _{min} (linear correction)	376 ± 16	365 ± 14	NS
QT _{min} (Bazett's correction)	398 ± 16	381 ± 12	0.02

Measurements taken at baseline and after phenylephrine (Phe) infusion, with and without atropine (ATR).

intervals were corrected with both Bazett's formula ($QT_c = QT/RR^{1/2}$) and Hodges et al's¹⁰ linear correction formula ($QT_c = QT + 1.75 [HR - 60]$).

QTd was calculated in electrocardiograms in which ≥ 9 leads were measurable. QTd is defined as $QT_{\text{max}} - QT_{\text{min}}$. Corrected QT dispersion (QT_{cd}) is defined as $QT_{\text{cmax}} - QT_{\text{cmin}}$. The intra- and inter-observer variability were 6% and 7%, respectively, for measurements of QTd.

Statistical analysis: All data were analyzed using the Statgraphics software package (STSC Software Publishing Group, Rockville, Maryland). The relation between variables were studied by Pearson's correlation and linear regression analyses. Differences between treatment groups were analyzed using the paired Student's t test. Results were expressed as mean ± SD. Differences were considered statistically significant if p was < 0.05 .

RESULTS

Baseline measurements: BP, HR, and electrocardiographic parameters at rest were similar at the start of both study visits (Table I). On the placebo study day, there were no changes in either hemodynamic or QTd parameters before and after infusion. Following atropine, baseline HR increased significantly but BP at rest was unchanged. This was paralleled by a significant reduction in QT_{max} and an increase in the Bazett-corrected QT_{max}. The linearly-corrected QT_{max} did not increase significantly. Atropine, however, did not appear to have any significant effect on either QTd or QT_{cd}.

Correlation between alterations in blood pressure and QT dispersion: PHENYLEPHRINE ALONE: Phenylephrine given in incremental doses caused a gradual increase in BP paralleled by a significant decrease in reflex HR. Both QT_{max} and the linearly corrected

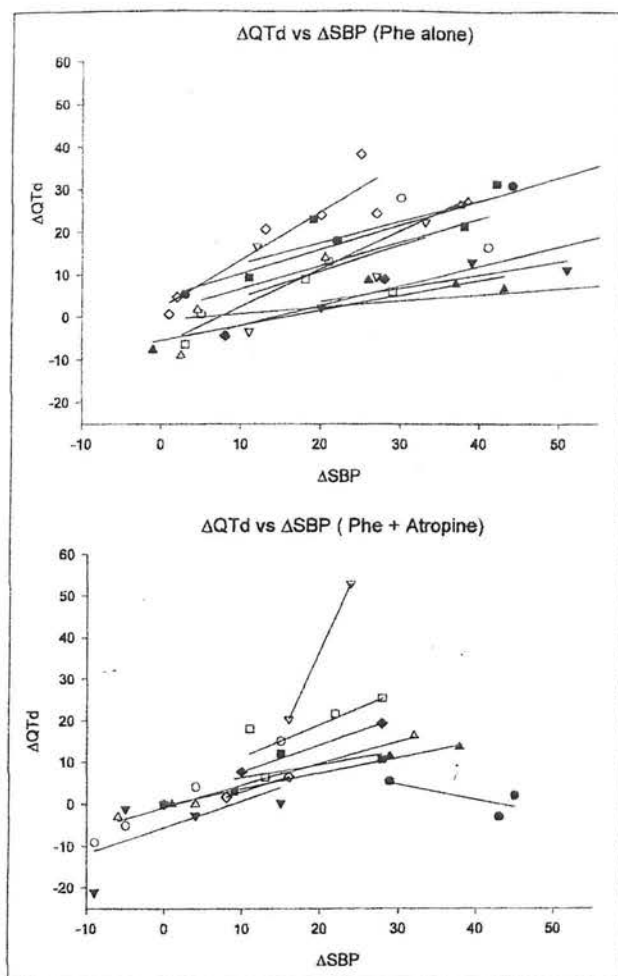


FIGURE 1. Individual data: changes in QTd (ΔQTd) in response to changes in systolic blood pressure (ΔSBP) following phenylephrine (Phe) infusion alone (top) and Phe + atropine (Atr) (bottom).

QT_{cmax} significantly increased, but the Bazett-corrected QT_{cmax} was not significantly affected by the changes in BP induced by phenylephrine (Table II). The relation between changes in BP and QT interval and dispersion indexes in response to phenylephrine are displayed in Figures 1 to 5 and Table III. It is clear that phenylephrine caused a significant increase in QTd and QT_{cd} and that this occurred in the presence or absence of atropine.

Changes in QTd (ΔQTd) and QT_{cd} (ΔQT_{cd}) appear to be significantly correlated with both changes in systolic and diastolic BP in a linear fashion. The relations between the variables, expressed as the slope of the linear regression line (e.g., $\Delta QTd/\Delta$ systolic BP), and their respective p values and correlation coefficients for each individual are displayed in Table III. Similar linear regression analysis made on the pooled data are displayed in Figures 1 to 3.

PHENYLEPHRINE AND ATROPINE: Following pretreatment with atropine, phenylephrine had no significant effect on either HR, QT_{max} , or QT_{cmax} (Table II). The previously demonstrated relation between the dispersion indexes and BP was still present and was

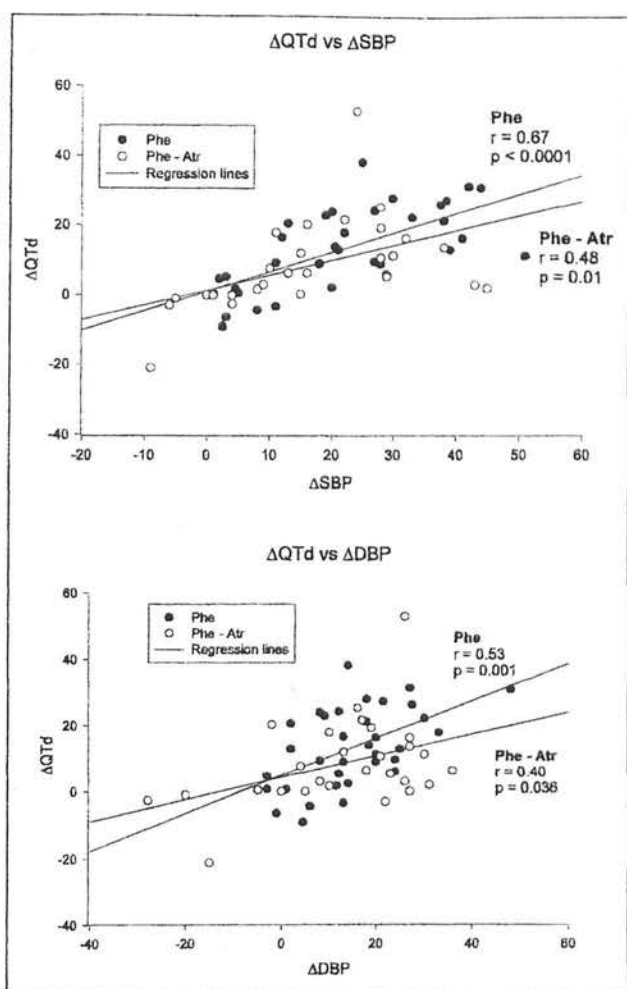


FIGURE 2. Pooled scatterplot: relation between changes in QTd (ΔQTd) and changes in systolic BP (ΔSBP) (top) and diastolic BP (ΔDBP) (bottom) in response to phenylephrine (Phe) \pm atropine (Atr). The correlation coefficient, r , and p value for each linear relation are displayed.

unaffected by atropine pretreatment. There were no significant differences in the slope, $\Delta QTd/\Delta$ systolic BP before and after atropine (mean difference -0.003 [95% confidence intervals (CI) -0.558 to 0.552] ms/mm Hg; $p = 0.9$). Similarly, atropine did not affect $\Delta QTd/\Delta$ diastolic BP (mean difference -0.271 [95% CI -1.060 to 0.517] ms/mm Hg; $p = 0.45$), $\Delta QT_{cd}/\Delta$ systolic BP (mean difference -0.404 ms/mm Hg [95% CI -1.066 to 0.258] ms/mm Hg; $p = 0.2$), or $\Delta QT_{cd}/\Delta$ diastolic BP (mean difference -0.383 [95% CI -1.214 to 0.449] ms/mmHg; $p = 0.32$).

DISCUSSION

In the present study, we found a significant positive correlation between changes in cardiac afterload and changes in all QTd indexes. Importantly, our atropine data showed that this relation was independent of HR changes or reflex vagal activity. Phenylephrine is a vasopressor, acting principally on the arterial bed. The increase in afterload is accompanied by a decrease in

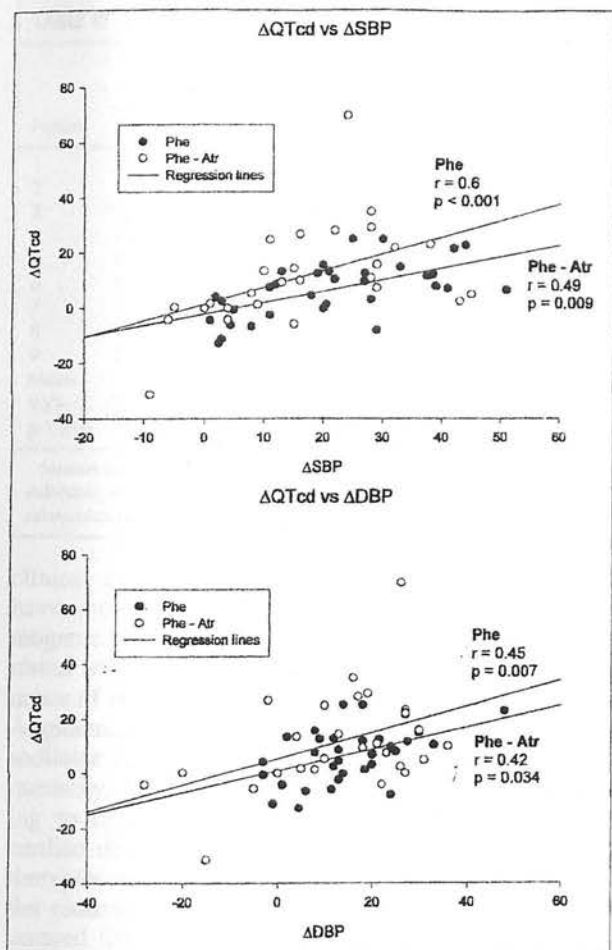


FIGURE 3. Pooled scatterplot: relation between changes in Bazett-corrected QTd ($\Delta QTcd$) and changes in systolic BP (ΔSBP) (top) and diastolic BP (ΔDBP) (bottom) in response to phenylephrine (Phe) \pm atropine (Atr). The correlation coefficient, r , and p value for each linear relation are displayed.

HR, resulting from reflex vagal activation. Pretreatment with atropine not only enabled us to assess the contributory role of vagal blockade on QTd, but also allowed us to control for HR. Due to the varying HRs, the effects of phenylephrine on Bazett's rate corrected QT intervals were significantly different from uncorrected values in our study (Tables I and II). As can clearly be seen, the QTmax values were not only significantly different between corrected and uncorrected values, but they moved numerically in opposite directions as a result of over-correction with the Bazett's formula.^{11,12} This has led many investigators to question the appropriateness of HR correction formulas such as Bazett's.

However, despite this, the relation between changes in QTd and changes in BP was unaffected by HR (Figures 2 to 3). Although it appears that acute cardiac afterload increases QTd, there does not appear to be any significant correlation with QTcmax (Figure 4). The impact of these major hemodynamic perturbations appears to be mainly on the QTcmin (Table II, Figure 5). The reduction in QTcmin with afterload is consistent with experimental data where stretching of

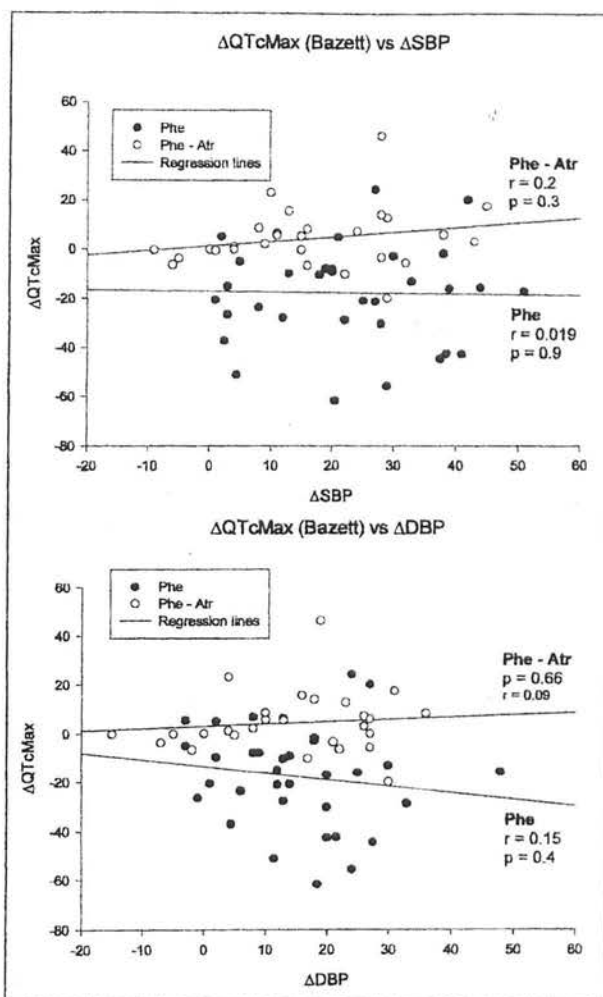


FIGURE 4. Pooled scatterplot: relation between changes in Bazett-corrected QTcmax ($\Delta QTcmax$) and changes in systolic BP (ΔSBP) (top) and diastolic BP (ΔDBP) (bottom) in response to phenylephrine (Phe) \pm atropine (Atr). The correlation coefficient, r , and p value for each linear relation are displayed.

the ventricular myocardium has been shown to shorten action potential duration.⁷⁻⁹

Afterload and QT dispersion: Our findings are consistent with the results of a recent study by Sun et al,¹³ who found a 22% increase (although nonsignificant) in dispersion of the QT interval following phenylephrine infusion in normal subjects. It is well established from experimental models that alterations in cardiac afterload may affect the action potential duration through mechanoelectrical feedback.⁷ From the law of Laplace, an increase in afterload should result in an increase in wall stress. Stretching of the ventricular myocardium has been shown to shorten the action potential duration in a number of studies.⁷⁻⁹ Importantly, however, these changes in effective refractory period may not be homogenous throughout the myocardium. Dean and Lab⁹ found that in an in situ pig heart model, there was a greater change in refractory period occurring at the apex compared with the base in response to an increase in load by aortic clamping.

The effects of afterload on QTd have important

TABLE III Effect of Changes in Blood Pressure on QT Dispersion: Individual Data

Patient	$\Delta QT_d/\Delta SBP$ (ms/mm Hg)		$\Delta QT_d/\Delta DBP$ (ms/mm Hg)		$\Delta QT_c/\Delta SBP$ (ms/mm Hg)		$\Delta QT_c/\Delta DBP$ (ms/mm Hg)	
	Phe (r)	Phe-Atr (r)	Phe (r)	Phe-Atr (r)	Phe (r)	Phe-Atr (r)	Phe (r)	Phe-Atr (r)
1	0.610 [0.61]	0.685 [0.98]	0.788 [0.60]	0.952 [0.98]	0.533 [0.80]	1.019 [0.99]	0.678 [0.78]	1.398 [0.96]
2	0.891 [0.98]	-0.190 [0.98]	1.700 [0.96]	-0.395 [0.93]	0.622 [0.98]	-0.226 [0.79]	1.169 [0.96]	-0.218 [0.36]
3	1.130 [0.84]	0.640 [0.68]	1.821 [0.88]	0.726 [0.40]	0.755 [0.85]	0.642 [0.45]	1.154 [0.77]	1.394 [0.76]
4	0.460 [0.98]	2.071 [0.95]	1.022 [0.99]	1.538 [0.90]	0.354 [0.99]	2.728 [0.95]	0.775 [0.98]	2.027 [0.90]
5	0.512 [0.81]	0.320 [0.64]	0.520 [0.80]	0.520 [0.70]	0.179 [0.28]	0.383 [0.55]	0.174 [0.26]	0.632 [0.62]
6	0.490 [0.81]	0.780 [0.75]	0.819 [0.80]	0.461 [0.20]	0.307 [0.79]	1.017 [0.75]	0.581 [0.89]	0.572 [0.19]
7	0.311 [0.86]	0.395 [0.96]	0.987 [0.95]	0.176 [0.99]	0.238 [0.85]	0.626 [0.99]	0.752 [0.95]	0.258 [0.96]
8	0.550 [0.74]	0.381 [0.99]	0.806 [0.82]	0.516 [0.96]	0.335 [0.47]	0.559 [0.98]	0.524 [0.56]	0.714 [0.90]
9	0.625 [0.98]	0.525 [0.98]	0.706 [0.99]	2.234 [0.62]	0.495 [0.99]	0.706 [0.98]	0.550 [0.98]	3.024 [0.62]
Mean	0.619	0.623	1.019	0.748	0.424	0.828	0.706	1.089
95% CI	(0.429-0.809)	(0.152-1.093)	(0.676-1.362)	(0.159-1.336)	(0.279-0.570)	(0.211-1.445)	(0.466-0.946)	(0.329-1.849)
p Value	<0.0001	0.016	0.0001	0.019	0.0001	0.015	0.0001	0.011

Relation between changes in systolic BP (ΔSBP) and diastolic BP (ΔDBP), and QTd are expressed in terms of the linear regression slope ($\Delta QT_d/\Delta BP$) for each individual, in response to phenylephrine (Phe) (\pm atropine [Atr]). r [in brackets] denotes the linear correlation coefficient. p < 0.05 denotes a statistically significant relationship between the 2 variables.

clinical implications. A number of previous studies have shown that an increase in afterload is arrhythmogenic.^{5,6} Furthermore, patients with conditions associated with an elevated afterload have an increased incidence of ventricular arrhythmias and sudden deaths.¹⁴⁻¹⁶ Angiotensin-converting enzyme (ACE) inhibitors, a vasodilator drug, in contrast, has been shown to reduce mortality, including sudden death.^{17,18} It is also intriguing to note that although QTd is increased in these cardiac diseases,^{1,2} the reduction in mortality by drug therapies such as ACE inhibitors is paralleled by a similar reduction in QTd.¹⁹ It is generally believed the increased QTd in these conditions occurs as a result of chronic changes in the myocardium, such as left ventricular hypertrophy.^{20,21} We believe that our study is the first to show that QTd may also be affected by acute changes in BP.

Vagal activity and QT dispersion: In our study, atropine reduced the resting QTmax (and QTmin) but increased the Bazett-corrected QT_cmax (and QT_cmin) significantly, an effect that has also been observed in other studies.^{11,22} However, the QT intervals were not significantly changed when linearly corrected. Changes in QT intervals are highly dependent on HR changes with QT interval shortening occurring as HR increases with atropine. As discussed in the previous section, the increase in Bazett-corrected QT_cmax is believed to be due to over-correction by the Bazett formula.^{11,12} As such, it remains uncertain whether vagal activity does indeed modulate ventricular repolarization; although some experimental data suggest that the cholinergic effects on ventricular repolarization are minimal,²³ others have found that atropine shortens the ventricular effective refractory period and QTmax interval during fixed atrial pacing.^{11,12}

QTd, unlike the QT interval, is not HR dependent (as reflected by the fact that we have found no discrepancy between our results with QTd, Bazett corrected QT_cd, or linear corrected QT_ld). In our study, QTd was unaffected by atropine. Our results are consistent with the observations made by Uemura et al.,²⁴

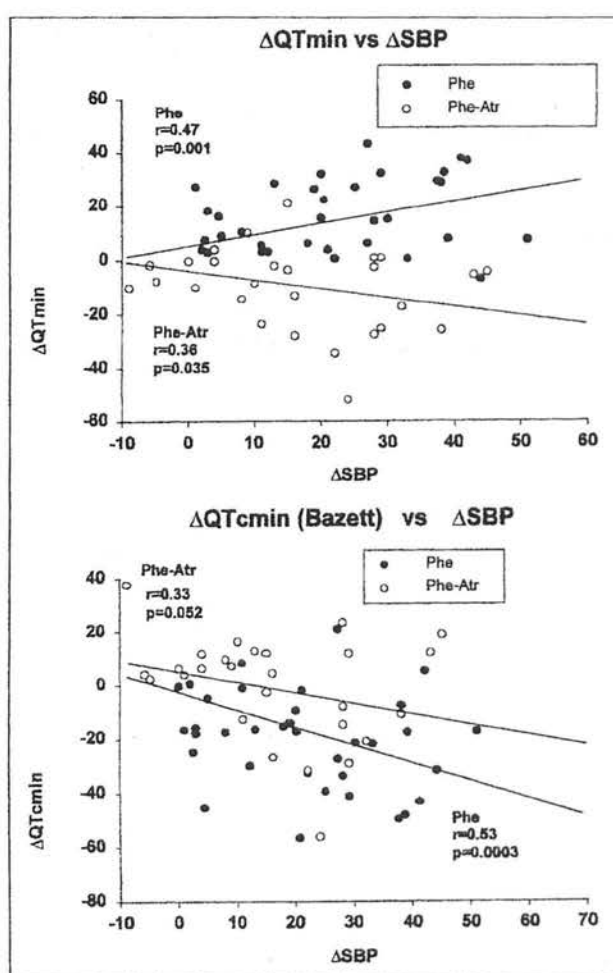


FIGURE 5. Pooled scatterplot: relation between changes in QTmin (top) and Bazett-corrected QT_cmin (ΔQT_cmin) (bottom) and changes in systolic BP (ΔSBP) in response to phenylephrine (Phe) \pm atropine. The correlation coefficient, r, and p value for each linear relation are displayed.

the only study that we know of that has directly examined the effects of atropine on QTd in man.

The reflex vagal response to phenylephrine has

been previously investigated by Kautzner et al.²⁵ This group used boluses instead of infusions of phenylephrine to assess if rapid vagal stimulation altered QTd. Boluses did not produce sustained increases in afterload. Hence, it is not surprising that Kautzner et al.²⁵ did not find any change in QTd. The finding that reflex vagal activity did not influence QTd is consistent with our observations.

Study limitations: First, although the study was designed primarily to assess the effects of afterload, any change in afterload will tend to affect preload as well. Although it is not possible to totally isolate the effects of one without changing the other, preload changes should be minimal because phenylephrine is an α -adrenergic agonist that acts primarily on arterial circulation.

Second, the technical difficulty of obtaining a precise and reproducible determination of the end of the T-wave measurement is well recognized. Care was taken to use consistent criteria to define the end of the T wave and to exclude U waves. We were careful not to miss any flat U waves that may have become more obvious following the phenylephrine infusion, because this may produce an artifactually short QT interval when a flat U wave becomes more obvious. Fortunately, this was not a major issue because the T and U waves were essentially normal and easy to measure at baseline in a cohort of normal, healthy subjects. Following phenylephrine infusion, both the T and U waves became more prominent, making measurements even easier. If the end of T wave in any lead was in doubt, the lead was excluded from analysis.

Third, a direct electrophysiologic effect of phenylephrine on the myocardium could not be entirely excluded. In isolated ferret Purkinje fibers, α -adrenergic stimulation was shown to induce early after depolarizations that could have been a trigger for arrhythmia initiation.²⁶ However, these effects are believed to be species specific, and have not been shown to occur in human myocardium.²⁷ Furthermore, early after depolarizations are HR dependent,²⁸ but in our study, the effects of phenylephrine on QTd were independent of HR. This means that a direct electrophysiological effect of α -adrenergic stimulation on QTd was unlikely. Finally, the study was performed in healthy normotensive subjects and not in patient groups. It remains to be seen if our observations also apply to the hypertensive population.

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